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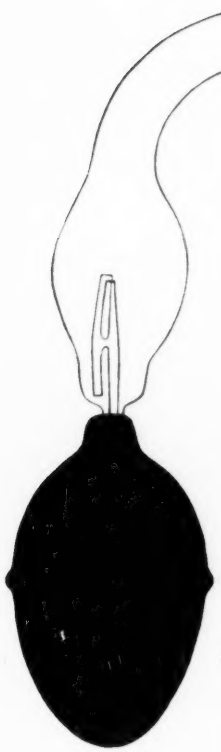

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1. Greene, G. G.: Kentucky M. J. *50*:8, 1952.
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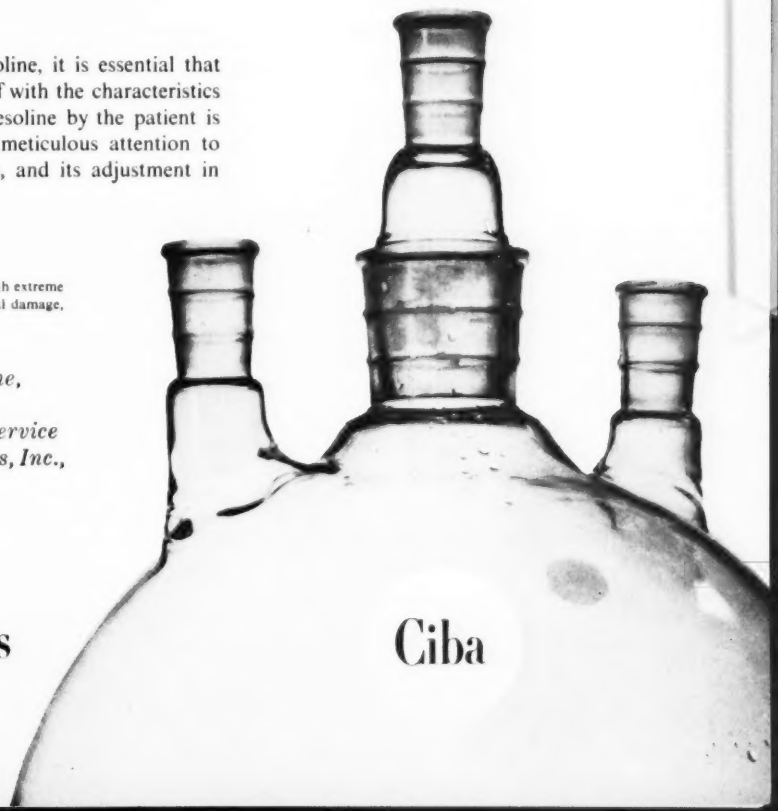

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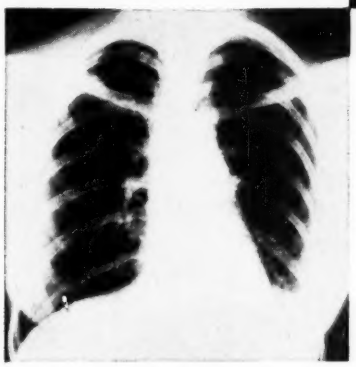

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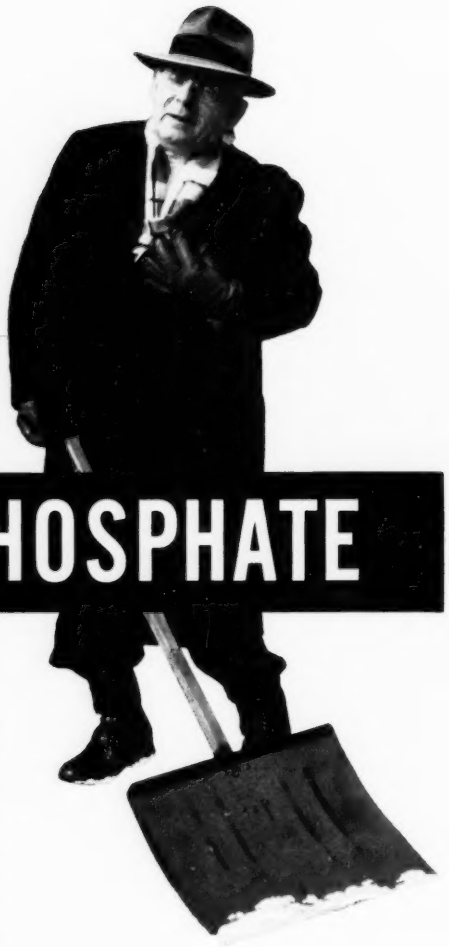

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MEASUREMENT OF HEPATIC BLOOD FLOW IN THE DOG BY THE BROMSULPHALEIN METHOD[1, 2]

By ATTIE YVONNE WERNER and STEVEN M. HORVATH

(From the Department of Physiology, College of Medicine, State University of Iowa, Iowa City, Ia.)

(Submitted for publication November 8, 1951; accepted February 5, 1952)

Estimation of hepatic blood flow by the bromsulphalein method appears to be based upon meager experimental evidence. Bradley, Ingelfinger, Bradley, and Curry (1) proposed a formula for the estimation of hepatic blood flow in man by measuring the hepatic extraction of bromsulphalein. Myers (2) compared the hepatic blood flow obtained by this method with the hepatic flow determined by urea formation. These two widely different methods yielded comparable estimates of hepatic blood flow. Sherlock, Bearn, Billing, and Paterson (3) have questioned the bromsulphalein method and have pointed out the considerable difficulties that can be experienced in the estimation of hepatic blood flow. Cohn and associates (4, 5) believed the high rate of extrahepatic removal of intravenously injected bromsulphalein invalidated the use of this substance for measuring hepatic blood flow. There is a need, therefore, for re-evaluation of the bromsulphalein extraction method of estimating hepatic blood flow. The reproducibility of hepatic blood flow measurements in a single animal and in a group of animals must be determined and the data so obtained subjected to analysis for statistical significance.

METHODS

Adult mongrel dogs, fasted 20 hours previous to experimentation, were anesthetized by the intravenous injection of 30 mg. nembutal/Kg. body weight. Under fluoroscopic guidance catheters were put through the right external jugular vein into a hepatic vein and the right ventricle. After the hepatic catheter was in position it was withdrawn slightly, still under fluoroscopic observation, to insure ease of sampling. Both the hepatic and right heart catheters were checked fluoroscopically after the bromsulphalein infusion was ended. In some instances catheters were placed in different hepatic veins. An indwelling needle or catheter was also placed in a branch of the femoral artery. All animals were heparinized, 5 mg./Kg. Blood pressures were recorded continuously by Statham strain gauges simultaneously from the femoral artery, right heart, and hepatic vein. A priming dose of bromsulphalein (BSP) was administered to raise the plasma level rapidly and to shorten the time required for equilibration. The priming dose of BSP was followed immediately by infusion of BSP at a constant rate for a period of 80 minutes.

In order to maintain a peripheral BSP concentration of less than 1 mg. per cent or greater than 1 mg. per cent, two rates or two concentrations of BSP infusions were given. The flow was controlled by the use of a Graubard Peterson flowrator. A sample of the infusion fluid was always taken for later analysis. Hepatic venous and peripheral arterial samples were withdrawn simultaneously at 10-minute intervals during the 40 to 80 minutes of each observation.

Five ml. of blood were collected in a dry syringe following preliminary flushing of the catheter by withdrawal of several ml. of blood. Hemolyzed samples were discarded. The hematocrit value was determined by the usual Wintrobe tube technique from samples of blood obtained at the midpoint of measurements of hepatic blood flow. Blood samples were also obtained from the femoral artery and right heart for the estimation of cardiac output by the Fick principle 60 minutes after the infusion had begun. The data on blood pressures and cardiac outputs are being reported in a separate communication. At the end of each period of observation incisions were sutured, 300,000 units of procaine penicillin administered and the dog returned to its cage. No animal was employed either for other observations or repeated hepatic observations until at least two or more weeks had elapsed.

BSP was measured colorimetrically in the macro unit of the Evelyn photoelectric colorimeter using filter 580. The calibration curve and the BSP solutions employed in all experiments were prepared from pure BSP powder (Hynson, Westcott, and Dunning).

One ml. of plasma was pipetted into an Evelyn colorimeter tube and the volume made up to 10 ml. with isotonic saline. After the initial "blank" reading, 2 drops of concentrated NaOH were added, the mixture well agitated, and a reading taken within one minute of adding the alkali. The BSP concentration was obtained from the galvanometer reading and the calibration curve. All determinations were made in duplicate.

[1] This work was supported by a grant from The Life Insurance Medical Research Fund.

[2] A preliminary report was given at the meeting of the Federation of American Societies for Experimental Biology, May 2, 1951.

TABLE I

Estimated hepatic blood flow in the anesthetized dog

The infusions in 49 observations** on 17 animals ranged from 0.63-3.25 mg. BSP/min. with a mean infusion rate of 1.73 mg. BSP/min. (S.E. 0.05), equivalent to 0.11 mg. BSP/Kg./min. (S.E. 0.03).

	Blood Flow		
	Range	Mean	Standard Error
ml./Kg./min.	16 to 111	42	2.9
ml./M^2/min.	495 to 3130	1140	78
ml./dog/min.	252 to 1878	690	52

**Each observation represents the mean value obtained from 3 to 6 sampling periods.

CALCULATIONS

When the infusion rate of BSP was such as to maintain a constant blood level (P), the infusion rate was considered to be equivalent to removal (R) of BSP and then the Fick principle could be applied. Estimated hepatic blood flow (E.H.B.F.) was calculated by the method of Bradley and associates (1) by the following formula:

$$\text{E.H.B.F.} = \frac{R}{0.01\ (P - H)} \times \frac{1}{1 - \text{hematocrit}},$$

where P represents the concentration of BSP in mg./100 ml. in the afferent hepatic blood and H the concentration in the efferent hepatic blood. Hepatic blood flow was calculated only from those 10-minute sampling periods where the level of BSP in the peripheral blood was constant or changing less than 0.0005 mg./ml./min. The BSP values utilized for the calculation of changing levels were interpolated midpoints on concentration curves of measured values. From three to five calculations were made from successive 10-minute sampling periods during each observation. The average of these 10-minute sampling periods was used to represent the E.H.B.F. of each observation. These averages were utilized in the calculations of the mean estimated hepatic blood flow.

RESULTS

The mean estimated hepatic flow was determined to be 690 ml./dog/min. in 49 observations on 17 animals. This was equivalent to 42 ml./Kg./min. or 1,140 ml./M^2/min. (Table I).[3] Equilibrium of BSP in the peripheral blood usually had occurred by the time the first sample was taken 40 minutes after infusion had begun.

The mean extraction of BSP by the liver for all observations was 34 per cent with a range of from 9 to 63 per cent. The percentage extraction of BSP was not significantly greater at lower than at higher peripheral BSP levels (Figure 1).

The livers of the dogs used in these observations were not weighed immediately after the observation period but autopsy records in this laboratory showed a liver weight averaging 3 per cent of the dog's body weight. The blood flow calculated on the basis of a liver weight of 3 per cent of body weight ranged from 57 to 358 ml./100 gr. liver/min. with a mean value of 140 ml./100 gr. liver/min., S.E. 8.2.

The reproducibility of E.H.B.F. in any one dog was determined. In comparison with the larger series of 49 observations on 17 dogs, the general reproducibility for any one dog was poor. However, in a few instances remarkably good agreement was obtained (Table II).

Catheters were placed in hepatic veins in the left lateral and right lateral lobes of the liver in order to estimate hepatic flow. Blood samples obtained from these two sampling sites were found to have approximately the same BSP concentration, and the calculated hepatic blood flows were similar.

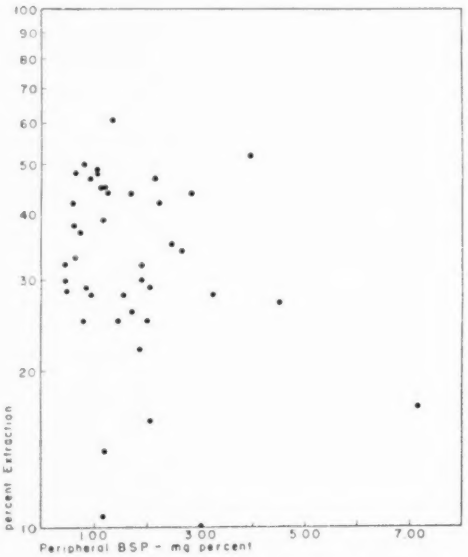

FIG. 1. THE RELATIONSHIP OF PERIPHERAL BROMSULPHALEIN VALUES TO THE PER CENT EXTRACTION BY THE LIVER

The trend line as obtained by the method of least squares is $y = -2x + 37.5$.

[3] A table of detailed data for this paper has been deposited with the American Documentation Institute, 1719 N Street, N. W., Washington 6, D. C.

TABLE II

Reproducibility of estimated blood flow measurement in the dog

Dog no.	Weight Kg.	No. of observations	Mean E.H.B.F. ml./Kg./min.	S.E.
46	12.4	7	43	4.2
47	13.3	6	26	2.2
48	19.2	4	33	4.4
44	15.2	4	50	6.5
32	16.9	3	43	8.1
55	20.3	3	40	6.9
56	19.0	3	49	8.7
36	18.0	2	37	0.1
43	14.6	2	33.5	3.5
52	18.8	2	23.5	6.5
53	14.9	2	28.5	2.5

These latter values fell within the statistical range reported above. The exact hepatic vein utilized to obtain hepatic blood samples does not appear to be of much consequence in explaining the variability of E.H.B.F.

Extreme variability was observed between any two compared 10-minute sampling periods. Although it was hoped the dogs would be maintained in a relatively constant state by the anesthesia, a mean range of change of 11 to 17.5 per cent was found (Table III). The lowest to the highest values during the three or more sampling periods were analyzed in order to determine the maximum in the variability. A mean difference of 29 per cent was found. The lowest E.H.B.F. did not necessarily occur in the 40/50-minute sampling period nor the highest in the 70/80-minute sampling period, nor vice versa, but could occur in any sampling period. The variations in the 10-minute blood samples were primarily due to variations in hepatic venous BSP concentration and to a lesser extent to changes in arterial BSP concentration.

An infusion of either a low concentration of BSP or of a high concentration of BSP was given

TABLE III

Comparison of estimated blood flows during various 10-minute sampling periods

Sampling periods compared	Mean difference per cent	Maximum range of difference per cent
40/50 min. to 50/60 min.	13	-35 to +62
40/50 min. to 60/70 min.	15.5	-38 to +57
40/50 min. to 70/80 min.	17.5	-48 to +57
50/60 min. to 60/70 min.	11	-40 to +47
50/60 min. to 70/80 min.	17	-54 to +38
60/70 min. to 70/80 min.	11	-45 to +36
period with lowest EHBF to period with highest EHBF	29	0 to +119

TABLE IV

Comparison of estimated hepatic blood flow when there was marked difference in the peripheral bromsulphalein concentration

	Peripheral BSP Concentrations	
	greater than 1 mg. per cent	less than 1 mg. per cent
No. of observations	27	18
ml./M²/min.	1040	1311
S.E.	85	100
t value	2.1	

in an attempt to produce a peripheral concentration of greater than 1 mg. per cent with high infusion rates or concentrations and less than 1 mg. per cent with low infusions. A comparison was made when the hepatic blood flows were divided into two groups based on these two levels of peripheral BSP concentration. The difference in blood flow was significant within the 5 per cent level (Table IV, Figure 2). It was possible to evaluate nine paired samples of data from dogs that were given both high and low infusions of

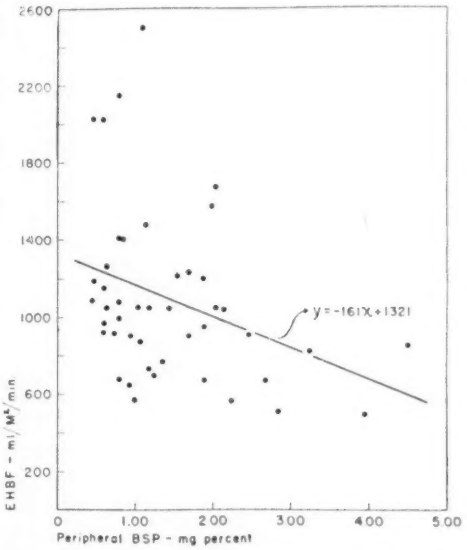

FIG. 2. THE RELATIONSHIP OF PERIPHERAL BROMSULPHALEIN VALUES TO THE ESTIMATED HEPATIC BLOOD FLOW

The trend line was obtained by the method of least squares, $y = -161x + 1{,}321$.

TABLE V

Measurements of hepatic blood flow in the dog

Investigators	No. of animals	No. of observations	Technique of measurement	Anesthetized	Total hepatic flow, ml./min.			Hepatic flow, ml./Kg./min.		Hepatic flow, ml./100 gr. liver/min.	
						Range	*Ave.*	*Range*	*Ave.*	*Range*	*Ave.*
Burton-Opitz (6, 7)	15	—	stromuhr	yes	portal vein	123.5–565	268	8.2–33.2	31.7		84
					hepatic artery	101.3–187	143.5	5.0–11.8	7.8	—	
					total		411.5				
Macleod and Pearce (8)	—	10	blood collected and measured	yes		250–534	390	26–85 (7 obs.)	44	64–144	95
Lipscomb and Crandall (9)	8	20	urea excretion	no		241–705	383	18–48	32	—	—
Grodins, Osborne	5	14			portal vein	52–400	147	5–37	15.5	—	—
Ivy and Goldman (10)	21	91	thermostromuhr	yes	hepatic artery	43–430	144	2–16	7.5	11–44 (21 obs.)	25
					total		291	total	23.0	total	—
Blalock and Mason (11)	10	13	blood collected and measured	no		266–612	415	18.7–39	28.6	58–112	86
Schweigk (12)	—	—	thermostromuhr	yes	portal vein	80–200	150				—
					hepatic artery	15–55	30	—	—	—	—
					total		180				—
Grab, Janssen, and Rein (13, 14)	6	—	thermostromuhr	no		157–520	330	18–33	26	43–70	58.5
Herrick, Mann, Essex					fasted	408–552	487	52–71	—		—
and Baldes (15)	1	3 days	thermostromuhr	no	fed	655–890	810	84–114	—	—	—
Soskin, Essex, Herrick, and Mann (16)	1	½ hour	thermostromuhr	yes		428–476	456	23–25	—	40–160 in a group of animals	100
Grindlay, Herrick, and		61	thermostromuhr	yes		252–620	407	16.8–30	23	—	—
Mann (17)	8	3		no		368–508	460	21–23.6	22.6	—	—
Werner and Horvath	17	49	BSP extraction	yes		252–1,878	690	16–111	42	57–358	140

BSP on the same day. The E.H.B.F. was calculated to be 1,146 ml./M²/min. with a peripheral BSP concentration of greater than 1 mg. per cent. When the peripheral BSP concentration was less than 1 mg. per cent, the E.H.B.F. was calculated to be 1,211 ml./M²/min. The mean difference in flow between the two peripheral levels was 65 ml. with a standard error of 1.08 and a t value of 0.60. Therefore, the slightly significant difference found on analysis of all samples was not confirmed when paired data were analyzed.

The data were subjected to further statistical analysis according to whether the infusion was greater or less than 0.10 mg./Kg./min. In 15 paired samples, the E.H.B.F. was 1,016 ml./M²/min. with an infusion of greater than 0.10 mg./Kg./min. When the infusion was less than 0.10 mg./Kg./min., the E.H.B.F. was 1,079 ml./M²/min. The mean difference in flow between the two infusion levels was 63 ml. with a standard error of 0.81 and a t value of 0.8.

DISCUSSION

Hepatic blood flow determined in anesthetized dogs by methods other than the bromsulphalein extraction method has averaged around 375 ml./min. (Table V). Grindlay, Herrick, and Mann (17) reported hepatic blood flow in the dog to average 23 ml./Kg./min. without anesthesia and 22.6 ml./Kg./min. with anesthesia. Habif and associates (18) measured hepatic blood flow in two patients before and during thiopental anesthesia and found a decreased hepatic flow in one and an increased hepatic flow in the other, while three other patients under cyclopropane anesthesia showed a reduced hepatic flow. The range of hepatic flow in anesthetized dogs was rather large as determined by one technique such as the thermostromuhr or by several methods such as direct collection of blood, stromuhr, and thermostromuhr. The mean flows ranged from 23 to 39.5 ml./Kg./min. and from 84 to 95 ml./100 gr. liver/min. Because of the wide range observed with these several methods it is probable that this range represents spontaneous variations in blood flow rather than variations in measurement due to the method employed. The values being reported for anesthetized dogs by the bromsulphalein extraction method are in excess of those obtained with other techniques (Table V). Lorber and co-workers (19, 20) have pointed out that BSP is found in the portal and hepatic veins and femoral artery after duodenal instillation of BSP suggesting that the amount of BSP normally excreted into the intestine via the bile must be considered in any evaluation of techniques using BSP. However, Owen (21) found no BSP in the peripheral blood after gastric or duodenal instillation of BSP. Cohn and associates (4, 5) have found that only 50 per cent of BSP infused into an eviscerate-hepatectomized-nephrectomized preparation remained 60 minutes later.

Preliminary experiments in this laboratory indicated that the extrahepatic removal of BSP was approximately 20 per cent, which is in agreement with the data of Brauer and Pessotti (22). Correcting the data obtained by the BSP method for this 20 per cent loss of the dye from the peripheral blood, the hepatic blood flow would be 24.5 ml./Kg./min. This is equivalent to 403 ml./dog/min., 665 ml./M²/min. and 82 ml./100 gr. liver/min. These corrected values are in close agreement with those obtained by other methods.

There was no difference in hepatic blood flow when the peripheral BSP concentration was greater or less than 1 mg. per cent. These results are in marked contrast to those presented for man by Sherlock and co-workers (3). These investigators pointed out that when the peripheral concentration of BSP was less than 1 mg. per cent, calculated hepatic blood flow appeared to be too great. This blood flow was almost twice that calculated when the peripheral BSP concentration was above 1 mg. per cent. The suggestion was made that at low levels of BSP concentration extrahepatic withdrawal might be a serious source of error. Ingelfinger (23) stated that the greatest error in calculating hepatic blood flow by the bromsulphalein method might occur at high peripheral BSP levels. The hepatic flow values reported here for the dog indicated that differences in flow were not due to different peripheral BSP levels. There was poor correlation between the peripheral BSP level and the E.H.B.F. in the dog, as Sherlock and associates (3) have shown to be true also for man. The percentage extraction of BSP was not significantly greater at lower peripheral BSP levels in the dog, unlike the correlation found in man (3).

The variations in hepatic blood flow determined by the BSP method are great. Because of these variations several 10-minute sampling periods must be used to evaluate blood flow. A single 10-minute sampling period is inadequate. The variation in flow which occurs may be explained either by a lack of sensitivity inherent in the method or by spontaneous variations in the normal state of the animals. Regardless of difficulties due to methodology, such as corrections needed for extrahepatic dye loss, the method appears valid within determined statistical ranges. Therefore, it is suggested that normal hepatic blood flow in dogs is subject to wide spontaneous variations. Corrected blood flow measurement is in agreement with hepatic flow studies determined by the thermostromuhr and other methods (7, 11, 16, 17).

The extreme variation in the hepatic flow even in the anesthetized dog may be due in part to the sphincter mechanism described in the dog (24), which may or may not be present in man. Several studies have indicated an intermittent and irregular rhythmicity of hepatic blood flow which Grindlay, Herrick, and Mann (17) showed may be due to alternate storage and discharge of blood by the liver.

Caution must be employed in any evaluation of hepatic blood flow measured by the BSP method. Since normal hepatic flow in the dog is subject to wide spontaneous variation, it is valid to evaluate changes in flow produced by drugs or by stimulation only when the changes observed are statistically different from control blood flow.

CONCLUSIONS

1. The estimated hepatic blood flow (E.H.B.F.) has been determined in 49 observations on 17 anesthetized dogs by the bromsulphalein (BSP) extraction method using hepatic vein catheterization. The mean E.H.B.F. was found to be 42 ml./Kg./min., S.E. 2.9. This is equivalent to 690 ml./dog/min., 1,140 ml./M^2/min. or 140 ml./100 gr. liver/min. These figures are too high apparently due to extrahepatic loss of BSP.

2. Deviations in hepatic flow determined from different sampling sites in the liver do not appear to exceed changes in hepatic flow from animal to animal or in the same animal on different occasions.

3. Differences in peripheral BSP concentration or differences in rates and concentrations of BSP infusion cause no significant difference in hepatic blood flow determinations.

4. Hepatic blood flow is subject to wide spontaneous variation. A single observation represents only the blood flow under conditions during that period of observation.

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THE CHANGES IN PLASMA POTASSIUM LEVEL AFTER EPINEPHRINE IN NORMAL HUMAN BEINGS AND IN PERSONS WITH EPILEPSY

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Biochemical studies directed toward finding a difference in the blood composition of normal and epileptic subjects have thus far been unfruitful. In the course of other studies at this laboratory (1), it was observed that the pattern of change in the plasma potassium level after an injection of epinephrine in a subject afflicted with epilepsy was different from that found in normal subjects similarly treated. An investigation was therefore undertaken to measure the changes in the concentration of plasma potassium, sodium and glucose at several time-intervals after an injection of epinephrine in epileptic patients and in normal human subjects. The results of this investigation showed that there was a significant difference in the pattern and extent of change in the plasma potassium level of epileptic subjects compared to normals subsequent to administration of epinephrine. There was no difference in the plasma sodium levels and only a probably significant difference in the extent of change in the plasma glucose level.

MATERIALS AND METHODS

Normal humans and patients with idiopathic epilepsy of both sexes were the subjects of this study. They were not told the nature of the tests to be performed except that several venous blood samples were to be taken for analyses. Eight of the subjects were patients of local physicians and five others were patients residing at Craig Colony, N. Y.[1] These patients had been taking prescribed amounts of one of the several anti-convulsant drugs and/or phenobarbital for one to several years. All of the epileptic subjects were well nourished individuals with no other complaints than their epileptic condition. They all were engaged in a variety of outdoor and indoor activities (three of the subjects were housewives; three were attending Junior High School; three were engaged in light mechanical jobs; and five of the subjects performed a variety of custodial duties). The normal subjects in this study were members of the staffs of the hospital and laboratories.

All of the tests were done in the morning with the subjects in a post-absorptive state. Immediately after the initial fasting blood sample had been obtained for the determination of baseline values, the subjects were given an intramuscular injection of 0.4 ml. of epinephrine (Adrenaline Chloride aq.; Parke, Davis & Co., 1:1,000). Blood samples were then obtained without stasis from one of the antecubital veins at four, 20, 60, and 120 minutes after the epinephrine injection. The blood was transferred to tubes containing 2 drops of heparin (Liqeamin, Organon, 10 mg./ml.) and immediately centrifuged. The plasma was immediately separated and aliquots taken for the various biochemical determinations. The plasma levels of sodium and potassium (m.eq./L) were determined with the aid of an internal standard lithium flame photometer using a dual-optical system of the type described by Berry, Chappell, and Barnes (2). Aliquots completely clear of hemolysis were used in these determinations. The plasma glucose levels (mg.%) were determined at first by the method of Somogyi-Schaffer-Hartmann (3) and then in most of the investigation by the photometric method of Kingsley and Reinhold (4).

RESULTS

The age, sex, number of grand mal seizures per month, and other information relevant to the epileptic subjects used in this study are shown in Table I. These subjects also experienced a number of petit mal attacks each month. They had been definitely diagnosed by their physicians as having idiopathic epilepsy. This was confirmed by the electroencephalograms [2] of all but one subject thus tested.

The mean per cent change (± S. E.) in the

[1] Grateful acknowledgment is made to Dr. Henry Brill, Medical Director, Craig Colony, N. Y., of the State of New York Department of Mental Hygiene, and the Hospital staff for their cooperation.

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TABLE I

Pertinent data of epileptic subjects used in tests

Patient	Age	Sex	Number of seizures per month†	E. E. G. report	Last attack before test (*wks.*)	Medication
Wi	62	F	2–4	E. posit.	1	Dilantin
Be	12	F	2–4	E. posit.	1	Dilantin
Ben	13	M	2–4	E. posit.	0.5	Dilantin
Hu	36	F	1	? E. posit.	—	Dil. and PhB.
Go	11	M	2	E. posit.	1	Dilantin
Wa	22	F	2	E. posit.	1	Dilantin
Ro	24	M	3–5	No test	1	Dilantin
Co	31	M	3–4	E. posit.	0.5	Dilantin
Ma	57	M	1	? Normal	4	None
Man*	28	M	2	E. posit.	2	Dil. and PhB.
Ron*	32	M	2	No test	2	Mes. and PhB.
Mu*	47	M	3–4	No test	1	PhB.
Ca*	21	M	2	No test	0.5	Dil. and PhB.
Ha*	42	M	2	No test	1	PhB.

* Residents of Craig Colony.
† Grand Mal seizures.
Dil. is Dilantin, Tid.
Mes. is Mesantoin, Tid.
PhB. is Phenobarbital, Tid.

plasma potassium level at four, 20, 60, and 120 minutes after epinephrine injection in the normal and epileptic groups of subjects is shown in Table II. It is evident from an inspection of these data that the plasma potassium levels were different after epinephrine in the two groups of subjects. In the epileptic group the mean plasma potassium level was found to be depressed from the initial level at four minutes after the epinephrine and persisted thus for a relatively prolonged time compared to the changes found in the normal group. Tests for statistical significance of the difference between the means of the two groups of subjects in each time-interval after epinephrine showed that the mean per cent change in the epileptic group was significantly different from the normals in all the periods except 20 minutes after epinephrine. It was in this period that the plasma potassium level in the normal group was maximally depressed from the pre-injection level.

TABLE II

*The change in plasma potassium levels in normal and epileptic subjects following an intramuscular injection of epinephrine**

Subjects	Minutes after epinephrine injection			
	4	20	60	120
Normals (*11*)	+1.6 ±1.1†	− 6.5 ±1.3	+1.0 ±1.4	+6.7 ±2.6
Epileptics (*15*)	−5.4 ±1.6‡	−10.2 ±1.7	−7.7 ±1.7‡	−2.8 ±1.6‡

* 0.4 mg. epinephrine injected immediately after fasting blood sample was taken.
† Mean per cent change (±S. E.) from the pre-injection mean potassium level.
‡ Value of "P" for difference between the means was significant (P = < 0.01 in each period).
Number of subjects in each group is in the parentheses.

TABLE III

*The change in plasma sodium levels in normal and epileptic subjects following an intramuscular injection of epinephrine**

Subjects	Minutes after epinephrine injection			
	4	20	60	120
Normals (*11*)	+0.8 ±0.4†	+0.8 ±0.5	+0.6 ±0.5	+0.5 ±0.5
Epileptics (*15*)	−0.4 ±0.7	+0.2 ±0.5	+0.3 ±0.5	−0.6 ±0.7

* 0.4 mg. epinephrine injected immediately after fasting blood sample was taken.
† Mean per cent change (±S. E.) from mean pre-injection sodium level.
"T" tests for difference between the means were not significant in all periods.
Number of subjects in each group is in the parentheses.

In Table III is shown the mean per cent change (± S. E.) in the levels of plasma sodium at the several time-intervals after epinephrine in the two groups of subjects. It is apparent that the concentration of sodium was unchanged from the respective initial levels in both groups of subjects. The difference between the means of the two groups was not statistically different.

The mean per cent change (± S. E.) in the levels of plasma glucose after epinephrine in the normal and epileptic groups is presented in Table IV. Inspection of these data show that both groups experienced a hyperglycemic response following the epinephrine injection. Although the pattern of change was the same in both groups of subjects, it is apparent that the mean per cent change was greater in the normal group. Tests for statistical significance of the difference between

TABLE IV

*The change in plasma glucose levels in normal and epileptic subjects following an intramuscular injection of epinephrine**

Subjects	Minutes after epinephrine injection			
	4	20	60	120
Normals (*11*)	+2.8 ±0.9†	+42.4 ±5.3	+49.0 ±6.7	+11.2 ±4.6
Epileptics (*15*)	+2.4 ±2.4	+23.7 ±3.9 0.008‡	+31.5 ±6.5	+ 1.1 ±4.8

* 0.4 mg. epinephrine injected immediately after fasting blood sample was taken.
† Mean per cent change (±S. E.) from the mean pre-injection glucose level.
‡ Value of "P" of difference between the means.
Number of subjects in each group is in the parentheses.

TABLE V

The initial plasma potassium levels and levels of maximum change after an intramuscular injection of 0.4 mg. epinephrine in normal and epileptic subjects

Normal subjects			Epileptic subjects		
Potassium (*m.eq./L*)		% change	Potassium (*m.eq./L*)		% change
Before epinephrine	After epinephrine		Before epinephrine	After epinephrine	
4.4	4.2	− 4.5	3.9	3.4	−12.8
4.5	4.0	−11.1	4.6	4.2	− 8.7
4.0	3.8	− 5.0	4.55	4.1	− 9.9
4.15	4.08	− 1.7	4.32	3.65	−15.5
4.15	3.98	− 5.3	5.0	4.16	−16.8
4.28	4.18	− 2.3	4.3	3.35	−22.1
4.83	4.6	− 4.7	4.9	3.85	−21.4
3.93	3.4	−13.5	4.28	4.0	− 6.5
4.95	4.38	−11.5	5.4	4.7	−13.0
4.60	4.13	−10.2	4.85	4.33	−10.7
4.27	4.18	− 2.1	4.05	3.44	−15.0
			4.48	3.87	−13.6
			4.53	3.95	−12.8
			4.56	4.0	−12.3
			3.9	3.4	−12.8
4.37±.10*	4.08±.09	−6.5†	4.51±.10	3.89±.10	−13.6

* Mean ± S. E.
† Mean.

the means of the two groups in each time-interval after epinephrine revealed that the difference was highly significant in the 20 minute period and probably significantly different ("P" equaled 0.06) in the 60 minute period.

It is evident that the change in plasma potassium level after epinephrine was the only one of the three (change in plasma level of potassium, sodium, glucose) which was significantly different in the epileptic group from that in the normal group. The data in Table V indicate that there was no difference between the post-absorptive plasma potassium levels in the normal and epileptic groups. It is also evident that there was a significant difference in the extent of maximum depression in the potassium level after epinephrine. These data therefore show that the initial potassium level could not have been a determinant factor in the difference between the two groups in the extent of plasma potassium change which was found after injection of epinephrine.

In Table VI are presented the mean per cent changes in the plasma potassium level of several epileptic subjects determined after epinephrine and again after a placebo injection of an equal amount of physiological saline. The two tests were performed in the same individuals about two weeks apart. Inspection of these data show that the placebo had not affected the potassium concentration. This is obviously different from the relatively large depression in the plasma potassium level found in these same subjects after epinephrine administration, and significantly changed at 20 and 60 minutes.

TABLE VI

The mean per cent changes in plasma potassium levels after a placebo injection and after an injection of epinephrine in the same epileptic subjects*

Injection	Minutes after injection			
	4	20	60	120
Placebo (7)	−0.6±2.3†	− 2.0±2.6	−1.4±2.7	0.0±2.7
Epinephrine (7)	−5.9±2.0	−12.2±2.4	−8.3±2.0	−4.8±2.5
	0.1‡	0.02	0.01	0.2

* 0.4 ml. of physiological saline was injected intramuscular as the placebo; and 0.4 mg. of epinephrine (1:1,000) was injected as the test agent.
† Mean ± S. E.
‡ Value of "P" of the difference between the means. Number of subjects in each group is in parentheses.

DISCUSSION

Several investigators have reported (5–7) in the past that both epinephrine and insulin administration in man and animals were followed by a fall in the plasma potassium level at approximately 20 minutes after their administration. The results presented in this paper showed that following an intramuscular injection of 0.4 mg. of epinephrine, there was a significant difference in the pattern of change in potassium level and the extent of fall in potassium level in subjects with idiopathic epilepsy from that found in normal human subjects. The level of plasma sodium was not affected by epinephrine administration in either group of subjects. It is of interest that Holler, Burton, Dury, and Keutmann (8) found, in a group of Addisonian subjects, an extensive and prolonged depression of the potassium level after the injection of 0.4 mg. of epinephrine, which generally resembled the pattern of changes in potassium level presented here in the epileptic group.

In a series of animal studies recently reported by Dury (9–11) it was shown that epinephrine induced a fall in plasma potassium level in normal, hypophysectomized, adrenalenucleated, and adrenalectomized rats. It was also shown that epinephrine-pretreatment protected adrenalectomized

rats from an experimental procedure which induced a fatal hyperkalemia in untreated adrenalectomized rats. These data indicated that epinephrine's effect upon the plasma potassium level did not require the mediation of the pituitary-adrenocortical system. The animal studies therefore indicate that epinephrine is related to a fall in plasma potassium level, probably due to a specific effect. The results presented here, showing a difference in the effect of epinephrine upon the plasma potassium level in normal and epileptic subjects, cannot, however, be explained from the data in this paper. However, a basis for explanation and speculation of this difference in potassium change in the normal and epileptic subjects is indicated by the results of a similar investigation in patients with Addison's disease (8) and in certain animal studies (9, 10). Holler and associates (8) found that the pattern of plasma potassium change after epinephrine in Addisonian subjects was similar to that reported here for epileptic subjects, but the extent of change in the former group was greater. In the animal studies it had been noted that the extent of fall in potassium level 60 minutes after epinephrine was greater in adrenalectomized than in normal rats. Whether the difference in plasma potassium change after epinephrine in normal and epileptic subjects is related to a difference in the functional status of the adrenal cortex or some other factor(s) must await further investigation. However, evidence admittedly of a preliminary nature which suggests this area of relationship is presented in Figure 1. The per cent change in the number of circulating eosinophiles is shown in the form of a scatter diagram for normal and epileptic subjects after epinephrine injection and after an injection of 25 mg. of ACTH. The eosinophiles were enumerated by the method of Randolph (12). Statistical analysis of the results showed that the mean per cent change was significantly lower in the epileptic group compared to the response obtained in the normal group. It is noteworthy that Aird and Gordon (13) recently reported that the frequency of epileptic seizures was decreased in epileptics treated with desoxycorticosterone.

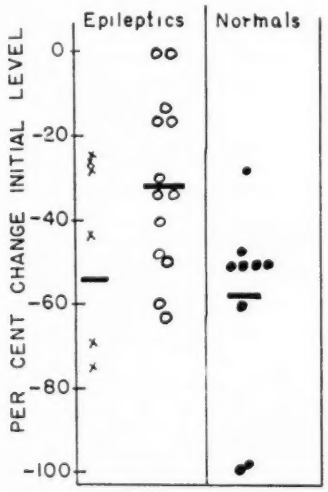

FIG. 1. PER CENT CHANGES IN NUMBER OF CIRCULATING EOSINOPHILES

Two hours after 0.4 mg. epinephrine, I. M., in epileptic (open circles) and normal (closed circles) subjects; and three hours after 25 mg. ACTH in epileptics (represented by X). Solid bar indicates mean change.

Statistical analysis of eosinophil data
(% change after epinephrine)

Normals	Epileptics	t	"P"*
−59±8†	−31±5	3.8	0.001

* Level of significance of difference between the means.
† Mean ± S. E.

SUMMARY

The changes in plasma levels of potassium, sodium, and glucose at four, 20, 60, and 120 minutes after an intramuscular injection of 0.4 mg. of epinephrine were determined in a group of normal human subjects and a group of subjects with idiopathic epilepsy.

It was shown that following the epinephrine administration there was a significant difference in the extent of plasma potassium fall and the pattern of change in the epileptic group as compared to that in the normal group. The plasma sodium levels were not affected in either group. A possible area which suggests a basis for an explanation of the results was discussed.

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RENAL CLEARANCES OF GRASS POLYSACCHARIDE: OBSERVATIONS ON GLOMERULAR POROSITY AND ON THE RELATION OF THIS FUNCTION TO PROTEINURIA IN RENAL DISEASE

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Dr. D. J. Bell, Biochemical Laboratory, Cambridge, drew our attention in 1948 to a fructosan polysaccharide (levan) prepared from Italian rye grass. It is readily soluble in cold water, yields levulose on acid hydrolysis and is not metabolized. At that time, the fructosan was believed to be a homogeneous substance with a molecular weight somewhat greater than that of inulin, so that the suggestion was made that it might replace inulin in the measurement of glomerular filtration rate. It has since (see below) been shown to be inhomogeneous. Thus, this report deals with the excretion characteristics of this dispersed polymer in normal unanesthetized dogs and in normal and abnormal human subjects.

METHODS

1. Preparation of the grass polysaccharide

The polysaccharide was isolated from a pure strain of Italian rye grass grown at the Hannah Dairy Research Institute, Scotland. The grass was cut in June 1948, and dried immediately. Details of the extraction method are given by Palmer (1).

2. Determination of the polysaccharide in plasma and urine

The polysaccharide was estimated in diluted urine and in deproteinized plasma filtrates by an adaptation of the inulin method of Harrison (2). Preparation of suitable protein-free plasma filtrates was at first difficult. Recoveries of the material from filtrates prepared by the use of cadmium sulfate and sodium hydroxide or of barium hydroxide and zinc sulfate were irregularly incomplete, ranging between 80 and 100 per cent. The Folin Wu method was not satisfactory in other respects. We turned to the acid zinc sulfate sodium hydroxide method of Somogyi (3); 100 ml. of acid zinc reagent were diluted to 212 ml. with water. This gave satisfactory recoveries at plasma dilutions of 1–20. Recoveries ranged from 96–102 per cent at plasma concentrations between 20 and 40 mg. per cent. Appropriate corrections were made for plasma and urinary blanks.

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3. Dog clearance techniques

(*a*) *Single injections:* Trained unanesthetized dogs were given 300 ml. of 0.3 per cent NaCl by stomach tube 30 minutes before the test. Test materials were injected intravenously at a rate of about 30 ml. per minute as 50 ml. of a solution containing 2 or 5 gm. each of polysaccharide and creatinine. Samples of blood and urine were collected at intervals, at first of five and 10 minutes and then at longer intervals over four to six hours. Plasma concentrations were calculated by interpolation to the mid point of each period of urine collection from a semilogarithmic graph of the determined concentrations, and these estimates were used in the calculation of plasma clearances.

(*b*) *Continuous infusion method:* A small plastic catheter was placed in the femoral vein and 30 ml. of priming solution, containing usually 1 gm. each of polysaccharide and creatinine and 4 gm. of mannitol, were injected rapidly. This was followed by a sustaining solution containing saline and given at a rate of about 1 ml. per minute. The bladder was rinsed with saline 15 minutes after completion of the priming injection; this was noted as zero time. Urine samples were collected at intervals of 10 to 15 minutes and combined with the saline bladder rinses (30–50 ml.). Samples of venous blood were collected near the mid points of each urine collection. This routine was varied in detail (concentration of polysaccharide, rate of infusion and intervals of collection) in some experiments.

4. Clearance techniques in human subjects

The methods, except for addition of polysaccharide to the priming and sustaining media in amounts of 3 and 5 gm., respectively, are those described elsewhere (4) for the determination of plasma clearances of mannitol (C_M) and p-aminohippurate (C_{PAH}). Some of the observations in patients include only the three periods of urine collection used in the measurement of PAH clearance at low plasma levels. PAH was omitted from most of the infusions used in normal subjects.

RESULTS

1. Experiments in dogs

(*a*) *Single injection method:* The ratio of polysaccharide (P) to creatinine (Cr) clearance (C) decreased progressively after injection. The poly-

TABLE I

Excretions of polysaccharide and creatinine in dog

Urine sample no.	Time after injection (*mins.*)	Urinary content (*mg.*)		Plasma concentration (*mg. per 100 ml.*)		Plasma clearance (*mg. per min.*)		Clearance ratio C_P/C_{Cr}
		P	Cr	P	Cr	P	Cr	
				(*a*) *Single injection*				
11-16-49								
1	10.0	1347	558	295	96	45.7	58.1	0.79
2	20.75	885	361	166	62	48.5	58.0	0.84
3	31.25	663	330	113	51	55.9	61.6	0.90
4	40.75	353	215	75	41	49.6	55.2	0.90
5	61.25	519	420	53	35	47.8	58.6	0.82
6	91.75	435	454	35	27.5	47.7	54.1	0.88
7	121.0	249	345	26	22	32.7	53.6	0.61
8	163.25	175	305	20	18.5	20.7	39.0	0.53
9	199.75	115	242	16	15.5	19.6	42.8	0.46
10	240.0	93	245	13.5	13.2	17.1	46.1	0.37
11	303.0	114	309	12.5	12.5	14.5	39.2	0.27
12	363.0	50	240	11.5	11.4	7.2	35.1	0.21
				(*b*) *Infusion method*				
11-3-48								
0	15							
1	25			61	22.7	24.0	39	0.62
2	35			60*	19.0*	17	40	0.43
3	49			59	17.4	14.5	44	0.33
4	59			57.5*	16.1*	14.9	46.6	0.32
5	69			55.5	15.5	12.3	45.7	0.27
6	79			52.5*	15.1*	11.4	41.3	0.28
7	89			48.5	15.2	11.0	39.7	0.28
3-24-48								
0	22							
1	32			64	18.6	27.5	43	0.55
2	42			56	18.5	24.6	46	0.53
3	60			47*	17.0*	20.3	37	0.54
4	70			41	15.3	20.6	41	0.46
5	79			38.5*	15.2*	48.7	43	0.44
6	90			36	15.0	17.8	42	0.43

Three experiments in dog 7–67, approximate weight 19 kg., the first by (*a*) the single injection method and the second by (*b*) infusion after a priming injection. Other measurements of filtration rate by the infusion method in this dog in late 1949 indicate a mean of about 55 ml. per minute, from which it seems that hydration and injection of polysaccharide do not disturb the rate of glomerular filtration. Contributing to the decrease of C_{Cr} in the latter periods of the first experiment were restlessness and repeated blood sampling. Plasma concentrations in (*a*) are obtained by interpolation from a smooth semilogarithmic curve of measured concentrations against time; interpolated concentrations in (*b*) are indicated by asterisk.

saccharide clearance (C_P) was about 80 per cent of the creatinine clearance (C_{Cr}) during the first periods of urine collection and decreased to less than 30 per cent by the end of six hours. The decline in the ratio was somewhat more rapid in dogs given smaller (2 gm.) than those given larger (5 gm.) amounts of polysaccharide. A representative experiment in a dog given 5 gm. of polysaccharide is shown in Table I(*a*).

(*b*) *Constant infusion method:* The aim of these experiments was to determine if the decrease in relative polysaccharide clearance observed after single injections could be related to the rapid fall in plasma polysaccharide concentrations. The experiments were carried on over one or two hours and at plasma concentrations ranging from 15–60 mg. per 100 ml. Plasma polysaccharide concentration was maintained at a constant level in some experiments and decreased or increased in others, as shown in Table I(*b*). The absolute and relative polysaccharide clearances could not be related to plasma polysaccharide concentration, to the direction or rate of change in this function or to urine flow. Instead, the decrease in rela-

tive C_P was a function of the time elapsed after the priming dose. Results of some 60 clearance periods (10 experiments in three dogs) indicate a range of the ratio C_P/C_{Cr} of 0.92 to 0.56 in the first 20 minutes after priming (approximate mean 0.74) and a range of 0.53 to 0.27 (approximate mean 0.34) 90 minutes later. The variability in the rate of change of the ratio with time is attributable in part to individual variability among the three dogs tested and possibly in part to the use of different lots of the polysaccharide in these experiments.

2. Human experiments

(*a*) *Normal subjects:* In these, as in the patients described below, the sustaining infusion was continued for about 90 minutes. Urine collections began 15 minutes after infusion of the priming dose and were made at intervals of 12–15 minutes. The ratio C_P/C_M was found to be constant in successive periods of urine collection (Table II), in contrast to the falling ratios found in dogs. The mean ratio in the 30 clearance periods was 1.12 (standard deviation ± 0.08). In our experience plasma mannitol clearance is about 10 per cent less than the simultaneous inulin clearance. Consequently C_P is at the level of inulin clearance or glomerular filtration.

(*b*) *Essential hypertension:* Observations were made in seven patients suffering from hypertensive disease of varying severity. In these, effective renal plasma flow (C_{PAH}) ranged from 156 to 491 ml., glomerular filtration rate ($C_M \times 1.1$) from 33–135 ml., filtration fraction from 0.25 to 0.37 and Tm_{PAH} from 21 to 77 mg. per minute, per 1.73 sq. M. body surface. The ratio C_P/C_M was constant in any patient over the period of observation. The mean of this ratio (0.98, standard deviation ± 0.07) is significantly different from that in normal subjects.

(*c*) *Glomerular disease:* In five patients suffering from acute or chronic diffuse glomerulonephritis, the mean of the ratio C_P/C_M was 0.76 (standard deviation ± 0.12). The ratio did not correlate with absolute levels of other renal function tests; in one patient (Table III, No. 1) it in-

TABLE II

Clearances and clearance ratios in successive clearance periods in human subjects

Clearance period no.	Normal		Essential hypertensive disease		Primary glomerular disease	
	Means	No.	Means	No.	Means	No.
1	1.17	5	0.98	7	0.63	9
2	1.10	5	0.98	7	0.60	9
3	1.07	5	0.96	7	0.65	9
4	1.11	5	0.99	7	0.70	4
5	1.08	5	0.96	7	0.70	4
6	1.08	5	0.96	7	0.74	4
Means	1.10		0.97		0.67	

Means and number of observations in successive clearance periods and means of these means of the ratio C_P/C_M in each of the groups of human subjects demonstrating that this function in human beings is not depressed as the time after the priming dose in prolonged clearances measured by the infusion method. The end of period 1 corresponds to 15 to 20 minutes after priming; each period is of 10 to 12 minutes duration so that elapsed time to the end of period 6 is about 80 minutes.

TABLE III

*Renal functional data in glomerular disease**

Diagnosis	No.	Date	R.P.F.	G.F.R.	F.F.	Tm_{PAH}	C_P/C_M	Periods
Ac. glom.-neph.	1	12-49	827	79	0.10	—	0.75	3
		1-50	827	93	0.11	—	0.67	3
		6-50	517	70	0.14	38	0.73	3
		7-12	673	89	0.13	41	0.74	3
		8-9	574	69	0.12	56	0.83	3
Chr. glom.-neph.	2	12-49	432	51	0.12	23	0.67	6
		1-50	228	26	0.11	—	0.64	3
Ac. glom.-neph.	3	12-49	597	60	0.11	61	1.07	5
Chr. glom.-neph.	4	5-50	322	50	0.16	32	0.81	3
Chr. glom.-neph.	5	9-50	—	6	—	0.1	0.80	6
Amyloidosis	6	1-50	545	91	0.17	81	0.70	6
Glomerulosclerosis	7	10-50	108	19	0.18	—	0.88	3

* Diagnosis (Ac. glom.-neph. = acute diffuse glomerulonephritis, Chr. glom.-neph. = chronic diffuse glomerulonephritis), renal functional data (R.P.F. = renal plasma flow from C_{PAH}, G.R.F. = glomerular filtration rate from $C_M \times 1.1$, F.F. = filtration fraction, Tm_{PAH}, mean ratio C_P/C_M) and number of polysaccharide clearance periods in patients with primary glomerular disease.

creased during recovery from acute glomerulonephritis. The ratio was depressed in one patient with renal amyloidosis (confirmed by biopsy) and one with intercapillary glomerulosclerosis. These observations are listed in Table III.

3. Observations on proteinuria

The decrease in the ratio C_P/C_M found in hypertensive disease and the still lower values of this function in patients with glomerular disease, in whom proteinuria is usually more severe than in hypertension, prompted us to examine the association between the ratio C_P/C_M and proteinuria. Proteinuria was measured during 12 or 24 hour periods by the Shevky-Stafford method immediately before or after the determination of relative polysaccharide clearance. The correlation coefficient of the ratio C_P/C_M (x) to proteinuria in gm. per 24 hours (y) was found to be -0.74 (standard error ± 0.23). This correlation coefficient is more than three times the standard error, from which it follows that, in these cases of renal disease, proteinuria and the ratio C_P/C_M are inversely associated.

DISCUSSION

The diffusion coefficient and sedimentation constant of the polysaccharide, assuming a partial specific volume of 0.65, yielded Ogston (5) an estimated molecular weight of 5,400. Dialysis for three weeks against water removed two-thirds of polysaccharide; studies on the residue indicated that its molecular weight was about 8,400. It would seem that the material was not homogeneous, but consisted of a large proportion of a polysaccharide with a molecular weight of about 5,000 and a smaller fraction of molecular weight about 9,000. More recently Palmer (1) obtained four fructosan fractions from Italian rye grass by serial precipitations from increasing concentrations of ethanol. The molecular weights of these as calculated from their glucose contents were 6,160, 5,380, 3,240, and 3,080. By the same method she found molecular weights of two fractions of dahlia inulin to be 7,290 and 6,800. Inulin has been considered to have a molecular weight of 5,100 (6). Thus, the polysaccharide we used is probably less homogeneous than dahlia inulin, to which it is similar in most of its properties; and its molecular weight is not very different.

In spite of these similarities, the clearance of this polysaccharide is notably different from that of inulin in the dog, in which creatinine and inulin clearance rates are identical. The ratio C_P/C_{Cr} in the dog is always less than unity and decreases as the experiment is prolonged. This inequality of C_P and C_{Cr} can hardly be attributed to tubular reabsorption of this inulin-like polysaccharide; the clearance ratio is independent of the direction of change in, or absolute level of plasma polysaccharide concentration and also of urine flow. Rather it seems that the polysaccharide mixture is, in part, not freely filterable through the glomerular capillaries. The decrease in the ratio with time can then be explained by assuming that some part of the polysaccharide mixture is perhaps filtered as freely as inulin, while another part can escape through perhaps only 20 per cent of the glomerular surface.

This assumption is based on the concept of glomerular porosity proposed by Yuile (7), and his demonstration that hemoglobin (molecular weight 68,000) in dogs passes through 3 per cent of the glomerular surface and myoglobin (molecular weight 16,400) through 20 per cent (8). Similarly, Marshall and Deutsch (9) have shown recently a rough correspondence between the molecular size of proteins and their clearances relative to creatinine in dogs. They also point out the importance of molecular asymmetry as a determinant of filterability. Thus, in dogs, ovomucoid (molecular weight 27,000) and ovalbumin (molecular weight 44,000) are cleared at similar rates. In human subjects, the passage of hemoglobin through the glomerulus has been estimated as occurring through 3 per cent (10) or 12 per cent (11) of the glomerular surface.

Lack of complete correspondence between molecular weight of proteins and their glomerular filterability seems to hold for fructosans. Taking Palmer's estimates of molecular weight, the freely filterable inulin is heavier than any fraction of the grass polysaccharide. Alternatively, using Ogston's estimates, and assuming the molecular weight of inulin to be 5,100, it seems that inulin is close to the limit of filterability through the dog's glomerulus, and that part of the polysaccharide exceeds this limit. On either basis, lack of complete filterability may result from inhomogeneity of the polysaccharide as to weight,

diameter and charge or from differences in elongation of the molecules. The observations in normal human subjects indicate that the clearance of the grass polysaccharide is substantially at the level of glomerular filtration, from which it seems that the human glomerulus is somewhat less retentive than that of the dog.

The 10 per cent decrease in the ratio C_P/C_M in patients with hypertensive disease can be explained by assuming that 10 per cent or more of the residual glomerular surface may have become damaged and thickened so that, while it transmits mannitol freely, it retains part of the polysaccharide. On this assumption one might expect that the ratio C_P/C_M would fall off in successive clearance periods as did the C_P/C_{Cr} ratio in the dog. We have no explanation why this did not occur. The observed decrease in the C_P/C_M ratio in hypertension, where some degree of glomerular damage can be anticipated, suggested that the ratio might serve as an index of the degree of glomerular disease. The low ratios observed in patients with diseases primarily affecting the glomeruli seem to justify this expectation. We envisioned use of the ratio as a clinical test of relative glomerular porosity. Unfortunately, a patient (Table II, No. 6) who had a history of hay fever developed mild asthma and severe urticaria during administration of the polysaccharide. Another who had had severe asthma, developed a very frightening asthmatic attack immediately after administration of the priming dose of polysaccharide. Consequently, the use of grass polysaccharide as a routine test for glomerular damage is unduly hazardous.

The observations on proteinuria bear on the debated point of glomerular versus tubular participation in the genesis of proteinuria. A decrease in the ratio C_P/C_M in renal disease could be of either glomerular or tubular origin. If it were tubular, one would have to make the rather bizarre assumption that the damaged tubule somehow newly acquires a capacity for active reabsorption of polysaccharide, so selective that it does not entail a similar reabsorption of such a close congener as inulin. This is unlikely. Much more likely is the possibility that tubular damage would result in passive back diffusion of substances from tubular fluid and, since mannitol diffuses more rapidly than polysaccharide, this would involve an increase rather than a decrease in the ratio C_P/C_M. We can therefore admit the assumption that the ratio C_P/C_M is a measure of glomerular function and that depression of this ratio is evidence of a glomerular abnormality which can be characterized as a decrease in glomerular porosity to molecules with the physical characteristics of the polysaccharide.

The rates of proteinuria in our patients with hypertensive and primarily glomerular renal disease show a significant inverse correlation with the function C_P/C_M. Hence, on the above assumption, the proteinuria is more probably due to glomerular than tubular disorder. It is paradoxical that passage of plasma protein through the glomerulus should seem to increase as porosity to polysaccharide (molecular weight less than 10,000) decreases. The explanation may lie in the physical characteristics of the substances. Thus, Brandt, Frank, and Lichtman (10) found that glomerular porosity to hemoglobin is not increased in proteinuric patients but may be decreased. The mechanism of a decreased porosity to molecules normally partially or wholly filterable, such as polysaccharide and hemoglobin, in the presence of increased proteinuria can perhaps be visualized if one likens the passage of the filterable molecules as akin to true filtration, and the escape of plasma protein as a leak in a filtering surface.

SUMMARY

Renal plasma clearances of a polysaccharide isolated from Italian rye grass with a mean molecular weight of about 5,400 were measured in dogs and human beings. The observations in dogs indicated that the material was not all freely filterable through the glomerulus. The ratio of polysaccharide clearance (C_P) to creatinine clearance (C_{Cr}) was less than unity and decreased in successive clearance periods, apparently because of the more rapid excretion of the more filterable component(s) of the polysaccharide.

In contrast, in normal human beings, the ratio of C_P to mannitol clearance (C_M) averaged 1.1, indicating that the polysaccharide was freely filterable with a renal clearance equal to that of inulin. The normal human glomerulus is apparently a less retentive filter than that of dogs.

Observations in patients with hypertensive disease yielded a mean C_F/C_M of 0.98, indicating that about 10 per cent of the residual glomerular bed had become impermeable to the polysaccharide, while still permeable to mannitol. In patients with primary glomerular disease still lower ratios were found. These were interpreted as resulting from decreased glomerular porosity and were considered to confirm by functional means, the severe anatomical changes which would be found in the glomerular capillaries in these conditions.

Assuming the ratio C_F/C_M to be a measure of relative glomerular porosity, glomerular porosity is found to decrease as proteinuria increases in hypertensive and primary glomerular renal disease. From this it seems to follow that the mechanism of such proteinuria is more probably glomerular than tubular.

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ADDENDUM

The rhizomes of the wild iris (*Iris pseudocorus*) contain a polysaccharide, irisin, which Ogston estimated had a molecular weight of 22,000–24,000 and which was apparently homogeneous in the ultracentrifuge. The polysaccharide was isolated by the method of Euler and Erdtman (12). When its clearance relative to creatinine was determined in dogs, we found that it behaved as did the polysaccharide from Italian rye grass. From this is seemed likely that this material, like grass polysaccharide, was also inhomogeneous.

Since allergy to iris is much less common than grass allergy, we had hoped that the iris polysaccharide might be adapted to clinical estimates of glomerular porosity. However, the ratios C_{Ir}/C_M in three normal human subjects were 0.73, 0.50, and 0.53; values of 0.72 and 0.65 were found in two patients with essential hypertension; in 11 observations in patients with glomerular disease, the ratios ranged from 0.29 to 0.65. The excretion of iris polysaccharide evidently does not reflect the interesting changes in glomerular porosity measured by grass polysaccharide.

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SUPPRESSION OF SODIUM EXCRETION BY THE COLON IN CONGESTIVE HEART FAILURE AND CIRRHOSIS OF THE LIVER DEMONSTRATED BY THE USE OF CATION EXCHANGE RESINS[1]

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Evidence for a hormonal disturbance in those diseases in which the accumulation of edema fluid is a major clinical manifestation has, until recently, been meager and the conclusions doubtful. Thorn and Emerson (1), in 1940, observed the exacerbation of ascites and edema during the premenstrual period in a patient with glomerulonephritis and in a patient with cirrhosis of the liver which suggests that gonadal hormones may at least affect the course of edema in these diseases. In 1942 Futcher and Schroeder (2), in their demonstration of the impaired excretion of sodium chloride in congestive heart failure, suggested that the cause might be increased venous pressure or renal anoxia or "an endocrine mechanism possibly involving the adrenal cortex."

In the last two years more substantial, although indirect, evidence for a hormonal disturbance in edematous states has been forthcoming. Parrish (3) found in four of 10 patients with congestive heart failure increased excretion of urinary corticoids which prolonged the life of adrenalectomized rats. Deming and Luetscher (4) report greatly increased sodium-retaining activity (by a bioassay method) in the urine of patients with massive edema due to nephrosis or heart failure. Albert and Smith (5), following the administration of ACTH, desoxycorticosterone, or cortisone to normal individuals, found in five subjects who developed marked peripheral edema all the hemodynamic changes of heart failure, except a decreased cardiac output.

The present investigation affords further evidence of a hormonal disturbance in certain diseases in which edema fluid accumulates. It is well known that desoxycorticosterone diminishes the excretion of sodium by the kidney and the sweat gland (6) and recently it has been demonstrated that desoxycorticosterone also diminishes the excretion of sodium by the salivary gland and the colon (7). There is evidence that the sweat and salivary glands in the patient who is accumulating edema act in a fashion which resembles excessive desoxycorticosterone-like activity (8, 9). This study was designed to ascertain whether the colon of the edematous patient also acts in a fashion which resembles excessive desoxycorticosterone-like activity.

The concentration of sodium in the sweat and saliva is normally of sufficient magnitude to permit measurement of a possible decrease. It is, however, difficult to document a decrease in the excretion of fecal sodium because the amount of fecal sodium is normally quite small and variable (10). In order to insure sufficient amounts of sodium in the stool to be able to quantify a difference between the normal individual and the edematous patient, a cation exchange resin was fed (11). For proper interpretation of the data, the mechanics of the action of the resin in relation to sodium in the lumen of the gut must be considered (7). The exchange of ions on the surface of the resin is almost immediate and the pattern of electrolytes on its surface is dependent on the number and nature of the electrolytes in the surrounding medium (12, 13). In the small intestine the resin is in equilibrium with a solution containing about 140 mEq of sodium per liter. When more sodium ions leave the lumen of the gut than enter, such as occurs in the colon, there is a net reabsorption of sodium. In proportion to the other ions present, there are fewer sodium ions available for the resin in the lumen of the colon

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than in the lumen of the small intestine and a new equilibrium is established in the colon between the sodium on the resin and that in solution in the colonic contents. The balance between the transfers of sodium across the colonic mucosa and the "attraction" of the resin for the sodium eventually determines how much sodium is retained on the resin and hence the amount of sodium appearing in the stool. The net transfer (*i.e.*, eventual reabsorption) of sodium across the colonic mucosa in the normal individual may thus be compared with the net transfer in the edematous individual by a comparison of the amounts of sodium removed by the resin in the stool.

The patients who were studied were selected as representative of the severest type of sodium retention. The criterion by which patients were selected was that the daily urinary excretion of sodium was negligible in relation to the dietary intake. Of course there are edematous patients who gain weight from day to day and who excrete more sodium than the patients studied. Their weight gain is correspondingly slower. For example, if a patient ingests 5 grams of sodium daily and excretes only 2, he would then regularly gain weight, but if the intake is reduced to 1 gram, the patient will no longer accumulate fluid and might even lose weight by diuresis. Presumably in patients who are able to excrete a moderate amount of sodium in the urine the excretion of sodium by the colon, salivary gland, and the sweat gland is not sufficiently impaired to demonstrate a difference from the rather wide range of normal values. Patients whose urinary sodium excretion was severely limited were therefore selected for study in order to demonstrate a difference from the normal.

PROCEDURES

A cation exchange resin was fed to two groups of patients. The first, considered the control group, consisted of patients who were suffering from a variety of chronic diseases without evident disturbance in their water balance. The second group consisted of patients who were actively accumulating edema fluid because of congestive heart failure or cirrhosis of the liver. Both groups received the same regular ward diet without the privilege of adding salt. The edematous patients never excreted more than 5 and usually excreted less than 1 mEq of sodium per day in the urine. The plasma sodium concentration in these edematous patients was essentially normal (135 to 140 mEq per liter) except for one patient (CER, Table I), whose plasma sodium ranged from 128 to 132 mEq per liter.

Forty-five grams of carboxylic resin in the ammonium form or in a combination of the potassium and ammonium forms (ratio of one part of potassium to two of ammonium) were fed daily to both groups. Stools were collected and analyzed for sodium, potassium, and nitrogen. To make certain that samples of stool were representative of the period of feeding resin, analyses were not begun until four days after beginning resin therapy. The study was conducted on the wards of a chronic disease hospital and it was quite difficult to insure complete stool collections. For this reason, fecal sodium and potassium were expressed in terms of excreted nitrogen. The daily fecal nitrogen was assumed to be the same for both groups of patients since they were on the same diet. This mode of expression avoids the errors of incomplete stool collections and erratic bowel movements but does contain the error of the variability in fecal nitrogen excretion (14). The latter error was thought to be smaller under the circumstances of the investigation. In order to use units which were in some way representative of the daily fecal excretion of sodium and potassium the data have been expressed in mEq of sodium or potassium per gram of nitrogen.

Stools were dried under infra red light. Triplicate aliquots of the dried feces were treated with concentrated sulfuric acid and ashed in platinum crucibles at 700° C. The ash was dissolved in hydrochloric acid and analyzed for sodium and potassium. Separate aliquots of the dried feces were analyzed for nitrogen and ammonium. All nitrogen values have been corrected for ammonium and represent ammonium-free nitrogen.

Sweat was collected in rubber gloves. The gloves and arms were prepared according to the procedure suggested by Leslie and Levin (15). The patient was attired in hospital pajamas and covered with two woolen blankets. The lower extremities were placed in a whirlpool bath at 42° C. After a 20 to 40 minute immersion in the bath the gloves were removed and the accumulated sweat poured into a vial.

Saliva was collected in the following manner. The mouth was first rinsed with distilled water and the subject was given paraffin to chew. The saliva collected during the first minute's chewing was discarded. Two separate samples were taken in the second and third minutes. The values reported are the means of the sodium content of these two samples.

Sodium and potassium were determined with an internal standard flame photometer (16). Nitrogen was determined by the micro-Kjeldahl procedure and ammonium was determined according to Summerson's modification of the Van Slyke and Cullen procedure (17).

OBSERVATIONS

In the control group of eight patients without evident disturbance in their degree of hydration who received carboxylic resin, the mean fecal so-

TABLE I

Fecal excretion of sodium and potassium during resin administration
Comparison of the normal with the edematous patient

Normal					Edematous					
Pt.	Resin form	No. obs.	Sodium	Potassium	Pt.	Diagnosis†	Resin form	No. obs.	Sodium	Potassium
			*mEq/gram N**	*mEq/gram N**					*mEq/gram N**	*mEq/gram N**
CNT	NH_4	9	25.3	65.4	FAG	CL	$K \cdot NH_4$	2	12.2	54.3
CHG	NH_4	10	26.3	42.7	HAM	CHF	NH_4	3	13.4	121.7
AST	NH_4	8	33.2	53.3	FAG	CL	NH_4	4	14.5	33.8
KRS	NH_4	7	38.0	53.5	STP	CHF	$K \cdot NH_4$	2	15.4	31.0
KRS	$K \cdot NH_4$	7	40.2	74.4	HEN	CL	NH_4	16	20.0	61.8
CRW	NH_4	6	52.8	90.0	POL	CHF	$K \cdot NH_4$	2	20.5	97.8
LST	NH_4	6	53.2	53.1	CER	CHF	NH_4	2	20.8	80.2
BRD	NH_4	3	59.2	144.0	OST	CHF	NH_4	1	22.1	38.2
CHE	NH_4	7	63.9	69.8	POL	CHF	NH_4	3	24.7	57.5
LST	$K \cdot NH_4$	7	64.2	61.3	KNS	CHF	NH_4	15	25.9	67.6
Mean			45.6	70.8	Mean				19.0	64.3
Standard deviation			±14.9	±29.0	Standard deviation				±4.8	±29.1

* Mean of stated number of observations.
† CHF, Congestive heart failure; CL, Cirrhosis of the liver.

dium excretion of 10 sets of observations was 45.6 ± 14.9 mEq per gram of nitrogen (Table I). In the group of eight patients who were accumulating edema fluid because of cirrhosis of the liver or congestive heart failure, the mean sodium excretion of 10 sets of observations was 19.0 ± 4.8 mEq per gram of nitrogen. The values for the fecal excretion of sodium were essentially the same in a given individual whether he was receiving the ammonium resin or the ammonium-potassium mixture (KRS, LST, FAG, POL, Table I). The data indicate that under similar conditions of diet and resin administration the net transfer of sodium from the lumen of the colon to the surrounding blood stream is greater in the edematous patient than in the normal. The resin is consequently less effective in retaining sodium in the stool in the edematous patient than in the normal patient.

The mean fecal excretion of potassium was approximately the same in the control group and the edematous group. The amounts of potassium excreted seemed to have little relation to the amounts of sodium excreted. In a given individual, however, somewhat more potassium was excreted in the stool when potassium was incorporated in the resin (KRS, LST, FAG, POL, Table I).

Sodium excretion by the sweat and salivary glands was measured in three patients of the group who were accumulating edema. Two patients were suffering from congestive heart failure and one from cirrhosis of the liver. In each patient the concentration of sodium in the sweat and saliva was much reduced as compared to normal values (Figure 1). In 10 samples of hand sweat in seven normal individuals taken at various times, the mean concentration of sodium was 39 mEq per liter, range 15 to 75 mEq per liter. In two patients accumulating edema fluid, the concentration of sodium in the sweat was 8 and 13 mEq per liter. In 30 samples of saliva taken at various times in nine normal individuals, the mean concentration of sodium was 35 mEq per liter, range 10 to 61 mEq per liter, whereas in the three edematous individuals the concentrations of sodium in the saliva were 8, 7, and 5 mEq per liter. The data are few but are in agreement with similar

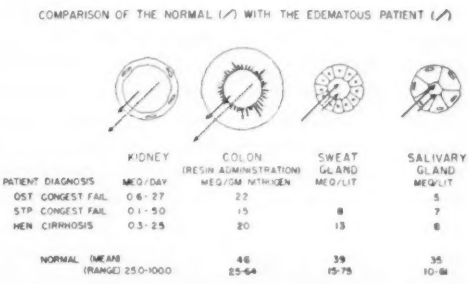
COMPARISON OF THE NORMAL (/) WITH THE EDEMATOUS PATIENT (/)
KIDNEY
COLON (RESIN ADMINISTRATION) MEQ/GM NITROGEN
SWEAT GLAND MEQ/LIT
SALIVARY GLAND MEQ/LIT
PATIENT DIAGNOSIS
MEQ/DAY
OST CONGEST FAIL 0.6-27 22 5
STP CONGEST FAIL 0.1-5.0 15 8 7
HEN CIRRHOSIS 0.3-2.5 20 13 8
NORMAL (MEAN) 46 39 35
(RANGE) 25.0-100.0 25-64 15-75 10-61

FIG. 1. NET SODIUM TRANSFER

data in the literature (8, 9, 18) and are presented only to have measurements in the same patient who fails to excrete expected amounts of sodium in the stool. The edematous patient, then, as compared to the normal, alters the transfer of sodium by various organs, the kidney, the colon, the sweat and salivary glands, in such a fashion as to limit the loss of sodium from the body.

DISCUSSION

The accumulation of edema in those patients who continually gain weight from day to day is due chiefly to the retention of sodium, chloride, and water ingested by the patient and not excreted by the kidney. The primary difficulty appears to be the retention of sodium. A disturbance in water excretion does not seem to be the basic difficulty. Although the patient with congestive heart failure or cirrhosis of the liver may eliminate a test dose of water more slowly than the normal individual (19–21), drinking large amounts of water daily does not aggravate the edema of congestive heart failure (22). Nor does it appear that the kidney has any difficulty in excreting the chloride ion as the edematous patient can excrete ammonium, potassium, or calcium chloride easily and sometimes with an increased amount of water (23). However, the urine of the patient who is actively accumulating edema fluid such as the type of patient reported here, contains only small amounts of sodium irrespective of the amount or form of sodium ingested. Under normal circumstances the kidney regulates the sodium content of the body by balancing the output against the intake. Why the kidney fails to do so in certain diseases is a point of controversy.

Many factors are known to affect the renal excretion of sodium. Alterations in filtration rate (24), renal arterial and venous pressure (25–27), oxygen tension (28), and plasma volume (29) are factors which have been investigated as individual entities and have been demonstrated to influence the renal excretion of sodium. The controversy resides in the relative importance of these factors and in the manner in which they play a role in the development of edema. The disturbed cardiovascular dynamics found in congestive heart failure are absent in cirrhosis of the liver, and the renal plasma flow and glomerular filtration rate are within normal limits (30). Nevertheless, in both these diseases where the patient is actively accumulating fluid there is evidence that several organs act to suppress the excretion of sodium. It is not to be inferred that organs other than the kidney contribute to any great extent to the accumulation of edema in congestive heart failure and cirrhosis of the liver. The data serve only to illustrate the fact that the suppression of the excretion of sodium in these diseases is a phenomenon which is common to various organs.

The fact that desoxycorticosterone has the ability to induce a similar disturbance in the transfer of sodium across the kidney, colon, salivary and sweat glands, suggests that the accumulation of edema in both cirrhosis of the liver and congestive heart failure is due, at least in part, to an excess of desoxycorticosterone-like hormone or to a deficiency of a possible antagonist. Desoxycorticosterone-like activity occurs as a normal physiological process when there are excessive losses of sodium from the body or when the dietary intake of sodium is sharply restricted (31–33) and is manifested by limiting the excretion of sodium from the body by several organs. The diminished excretion of sodium found in diseases where there is active accumulation of edema fluid resembles the normal mechanism for the conservation of salt. The question is why the normal mechanism for limiting the escape of sodium from the body goes awry in these diseases. It is not known whether the biochemical stimulus is the same for the normal conservation of salt as it is in congestive heart failure and cirrhosis of the liver or whether it is the same or different for these diseases. It is clear that in these edematous states four organs, across which the transfer of sodium can be measured, act in concert to limit the escape of sodium from the body. The accumulation of edema in these widely divergent clinical states would seem then to be due in part to a disturbance in hormonal balance.

CONCLUSIONS

With the use of cation exchange resin it has been demonstrated that the colon of the edematous patient retains sodium to a greater degree than the colon of the normal subject. This suppression of sodium excretion is part of a generalized phe-

nomenon involving the kidney, sweat gland and salivary gland. The inference is drawn that the accumulation of edema in congestive heart failure and cirrhosis of the liver is at least partly mediated through a disturbance in the hormonal control of sodium excretion.

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THE URINARY EXCRETION AND THYROID UPTAKE OF IODINE IN RENAL DISEASE[1]

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Radioactive iodine has proved itself a most useful tool in the assessment of thyroid function and since its introduction in 1938 by Hertz, Roberts, and Evans (1) a voluminous literature on this subject has developed, much of which has been well summarized by Skanse (2) in his monograph.

Although various workers have noted that there is a delayed excretion of the isotope in renal disease which probably invalidates any measurements of urinary excretion of radio-iodine or calculations derived from such measurements as a means of indirectly assessing thyroid function, relatively little investigation of the effect of renal lesions on the more direct measurements of thyroid function with radio-iodine has been carried out. McConahey, Keating, and Power (3) have noted an apparently decreased rate of accumulation of radio-iodine by the thyroid gland, a finding generally indicative of depressed thyroid function. It thus seemed of interest to further investigate this problem to see firstly if renal disease in any way invalidates the use of tracer doses of radio-iodine as a diagnostic aid in assessing thyroid function, and secondly, with the aid of the isotope, to study some phases of iodine metabolism and utilization in subjects with renal disorders.

SUBJECTS AND METHODS

Patients with kidney disease were chosen only when they showed obvious signs of renal failure—elevated blood urea nitrogen, gross albuminuria and poor urinary concentrating power. These subjects are listed in Table I. A control group composed of normal persons or patients not known to have any dysfunction of either kidneys or thyroid is similarly listed in Table II. In both groups special care was taken to select subjects who had not previously had organic iodine compounds, *e.g.*, priodax or lipiodol, and who had not had any inorganic iodine medication for at least two months previously. Observations were made on the subjects after an overnight fast of at least 14 hours and they continued without food for the first six hours of the experimental period.

The fasting subject was required to void completely, and immediately afterwards was given 100–125 microcuries of carrier free radioactive iodine orally. Two to two and one-half hours later when absorption of the radio-iodide was probably complete, the patient again voided, a blood sample was obtained and the time noted. Counts over the thyroid were then begun and midway through the counting period, a second blood sample was taken and the time noted. An accurately measured time interval of approximately two hours was allowed to elapse whereupon the patient again voided and blood samples and thyroid counts were repeated.

By these procedures it was possible to determine the clearance of radio-iodine from the plasma by the kidneys and the thyroid. A further blood specimen was obtained 26 hours after the administration of the tracer dose.

The quantity of radio-iodine present in the thyroid at any given time was expressed as a percent of the dose given and was determined by direct counts 6 inches above the thyroid isthmus, using a shielded collimated Geiger tube (4) and comparing the counts with similar ones over a standard solution of radio-iodine in a glass container. At least 1,000 counts were obtained in each instance. Background radiation was corrected for by subtracting counts obtained 6 inches over the middle of the thigh. Thyroid counts were made at two, four, 26, 50, and 75 hours after the ingestion of the dose.

Blood samples were collected in heparin or oxalate and the plasma immediately separated. Protein bound iodine estimations were made on one of the specimens, generally the first one taken, using the method of Barker (5), which was also found satisfactory for the determination of urinary iodine. The plasma concentration of I^{131} was determined on each sample of plasma, using the principles of the method of Barry (6) in which the radio-iodine together with a controlled weight of added carrier is precipitated as insoluble palladium iodide and counted. Duplicate 1 ml. aliquots of plasma were precipitated with 3 ml. 8% trichloroacetic acid. The samples were centrifuged and 2 ml. of supernatant removed; to this was added 1 ml. of 1.5% KI followed by an excess of palladium chloride. The resulting precipitate of palladium iodide was filtered off by means of a Tracerlab E-8A precipitation apparatus, dried and its radioactivity measured. The urine passed during the clearance period was similarly treated. For each aliquot at least 1,500 counts were scaled in determining the counting rates, which in

[1] The subject matter of this paper is part of a thesis submitted by Dr. Hughes to the Faculty of Graduate Studies, University of Manitoba in partial fulfillment of the requirements for the degree M.Sc. (Med.)

TABLE I

Clinical details of patients with renal disease

Cases	Age	B.U.N.	Plasma creatinine	Creatinine clearance	P.B.I.	Diagnosis
		mgm.%	*mgm.%*	*ml./min.**	*μgm.%*	
M. W.	50	38	1.1	52.6	7.8	Kummelstiel-Wilson's disease
F. B.	56	34	1.3	56.5	4.7	Bilateral pyonephrosis
H. A.	53	66	3.9	22.0	4.2	Chronic nephritis
E. M.	25	23	2.0	48.8	4.0	Sub-acute nephritis
M. R.	37	158	9.9	7.3	—	Polycystic kidneys
B. S.	22	49	3.9	20.6	4.7	Chronic nephritis
A. H.	37	23	1.4	86.9	—	Kummelstiel-Wilson's disease
H. A.	75	48	2.1	24.4	4.2	Nephrosclerosis
C. S.	52	21	1.1	87.0	3.3	Chronic nephritis
E. P.	74	34	1.4	37.5	4.9	Kummelstiel-Wilson's disease
E. V.	74	31	1.6	30.4	3.7	Nephrosclerosis
B. P.	37	25	1.2	69.3	4.9	Kummelstiel-Wilson's disease

* Standardized to body surface area of 1.73 sq. m.

duplicate samples agreed within 5%. A suitable aliquot of the original dose of I^{131} was subjected to a similar precipitation and its radioactivity measured. This formed the reference standard for the plasma and urine samples. All radioactive measurements were made with Tracerlab TGC-1 Geiger tubes (2 mgm./cm.^{2} windows) and a Tracerlab 64 scaler. Measurements of urine radioactivity were made until the asymptotic amount had been excreted. From the urine excretion curves one was able to derive certain values described by Keating, Power, Berkson, and Haines (7), namely, the disappearance rate of radio-iodine from the blood and the renal and extra-renal disposal rates of the radio-iodine.

Renal clearance of radio-iodine was computed using the conventional formula UV/P. However, as the plasma level of I^{131} falls in an exponential manner with time, a logarithmic mean concentration was calculated using the formula $\frac{P_1 - P_2}{\ln P_1 - \ln P_2}$ as suggested by Keating and associates (8). Clearance values were standardized to a body surface area of 1.73 sq. m. As a measure of the glomerular filtration rate endogenous creatinine clearance for the two hour clearance period was determined. Endogenous creatinine clearance has been shown to follow inulin clearance fairly closely (9) and thus will approximate the glomerular filtration rate. It was also standardized to a body surface area of 1.73 sq. m.

From the glomerular filtration rate and the renal clearance of radio-iodine the percentage of the filtered I^{131} reabsorbed by the kidney tubules was calculated.

The rate of clearance of I^{131} from the plasma by the thyroid gland was obtained by dividing the percentage of the dose accumulated per minute during the two hour clearance period by the mean plasma concentration (percent dose/ml.) during this period.

The plasma concentration of stable inorganic iodide during the clearance period was measured indirectly, as the direct measurement of physiological amounts of stable iodide in plasma is technically difficult compared with its measurement in urine. The procedure was similar to that of Stanley (10) by which it is assumed that radioactive and stable iodine are handled indiscrimi-

TABLE II

Clinical details of normal subjects and patients without renal disease

Cases	Age	B.U.N.	Plasma creatinine	Creatinine clearance	P.B.I.	Diagnosis
		mgm.%	*mgm.%*	*ml./min.**	*μgm.%*	
J. J.	51	—	1.1	114	—	Spontaneous pneumothorax
H. C.	43	—	0.7	108	—	Fractured femur
A. P.	71	—	1.1	83.3	—	Fractured humerus
M. N.	46	—	—	—	—	Atopic dermatitis
A. R.	25	—	0.8	114	5.0	Normal
E. C.	32	16	1.0	95	5.0	Normal
S. C.	37	12	1.1	107	5.2	Methyl hydrate poisoning—no kidney damage
C. N.	26	—	0.7	125	6.2	Normal
W. P.	26	—	0.9	124	4.5	Thumb injury
J. H.	30	—	0.9	124	4.0	Pneumonia
G. F.	21	—	0.9	146	4.2	Convalescent typhoid
D. S.	21	—	1.1	118	3.5	Normal

* Standardized to body surface area of 1.73 sq. m.

TABLE III

*The renal clearance and urinary excretion of I^{131}**

Subjects	Renal clearance		48 hour excretion		Renal excretion rate		Extra-renal disposal rate	
	no.	*ml./min.*	*no.*	*% dose*	*no.*	*%/hour*	*no.*	*%/hour*
Controls	11	31.4±2.0	9	78.3±3.5	9	10.2±1.0	9	3.6±0.8
Renal disease	11	5.5±1.2	8	41.7±7.5	7	1.6±0.3	7	1.1±0.3
P values	<.01		<.01		<.01		<.01	

* Mean values ± standard error.

nately by any organ. Thus at any given time the ratio of each in urine and plasma will be the same; and thus knowing the urinary concentrations of I^{127} and I^{131}, and from the determination of the mean plasma concentration of I^{131}, the plasma level of I^{127} is readily calculated. Likewise by assuming the thyroid is similarly non-selective towards iodine isotopes, the amount of iodine picked up per unit time by the gland can be calculated from the known urine or plasma concentrations of the two isotopes and the known rate of uptake of I^{131} by the gland. By this means the plasma level of I^{127}, the hourly uptake of I^{127} by the thyroid, and the hourly filtration and resorption of I^{127} by the kidneys during the two hour fasting clearance period were calculated. It has been shown that the distribution of I^{131} throughout the body requires some six hours (11). However, this need not invalidate the calculation so long as the plasma reaching the kidney and thyroid have the same levels of radio-iodine and the plasma level is falling in an exponential manner. The above calculations assume that all the urinary iodine is in the form of iodide. It has recently been shown by Rall (12) that organic iodine compounds may be excreted in urine, but in the euthyroid individual they comprise a very small portion of the total urine iodine and hence the resultant error in the calculated plasma iodide level should be small, and it is presumed in this study that the urine of individuals with renal disease likewise does not differ in this respect.

The percentage resorption of chloride and sodium loads by the renal tubules was estimated on some subjects. Sodium was measured by flame photometry and chlorides by the method of Shales and Shales (13) in plasma, and by the Volhard-Arnold method in urine (14).

RESULTS

(*A*) *Results on the urinary excretion of radio-iodine in kidney disease*

The renal clearance of radio-iodide by 11 patients with kidney disease and by 11 control subjects is shown in Table III. Also shown in this table are the 48 hour urinary excretions of the isotope and the calculated renal excretion rates and extra-renal disposal rates of radio-iodine, these latter two values being derived according to the principles of Keating and co-workers (7). All values were markedly lower in the patients with kidney disease including the calculated extra-renal disposal rate of I^{131}. This latter value approached the levels found in hypothyroidism and if assessment were made on this basis only it might be concluded that the thyroid glands of a person with renal dysfunction were underactive.

The level of the isotope in the plasma 26 hours after administration of the dose is shown in Table IV and further illustrates that there is considerable retention of radio-iodine. The mechanism of the poor renal clearance is demonstrated by other values presented in this table which indicate that not only fasting level of stable iodide I^{127} in the plasma is considerably greater than the level in the control subjects, but in addition the

TABLE IV

*The renal filtration and reabsorption of I^{131} and I^{127} under fasting conditions. The 26 hour plasma level of I^{131}; the fasting I^{127} plasma level**

Subjects	No.	% dose filtered (*per hr./ 1.73 m.²*)	No.	μgm. I^{127} filtered (*per hr./ 1.73 m.²*)	No.	% reabsorption of filtered I^{131}	No.	Reabsorption of I^{127} (*μgm./hr./ 1.73 m.²*)	No.	26 hour plasma I^{131} (*% dose/ 100 ml.*)	No.	Plasma I^{127} (*μgm./ 100 ml.*)
Controls	11	24.2±2.4	7	13.7±5.8	11	72.9±1.5	7	9.9±6.0	6	0.027±.006	7	0.17±.005
Renal disease	11	12.1±2.9	6	28.2±8.2	11	85.9±3.1	6	23.2±7.3	6	0.210±.012	6	2.20±0.50
P values		<.01		<.01		<.01		<.01		<.01		<.01

* Mean values ± standard error.

TABLE V

Percentage reabsorption of tubular loads of chloride and sodium under fasting conditions

Controls			Renal disease		
Case	Cl	Na	Case	Cl	Na
A. R.	99.8	99.1	E. P.	98.6	98.2
G. F.	99.1	98.4	E. V.	98.2	98.7
D. S.	99.7	99.5			

defective clearance of radio-iodide from the plasma is due not only to a decreased rate of glomerular filtration but also because the tubular reabsorption of the filtered I^{131} is significantly higher in those with renal disease. Despite depressed glomerular filtration rates, when the amounts of filtered and reabsorbed stable iodide I^{127} were calculated, it was found that under the fasting conditions of the experimental period considerable more iodide was filtered per unit time by the diseased subjects. This was due to the high plasma levels of iodide

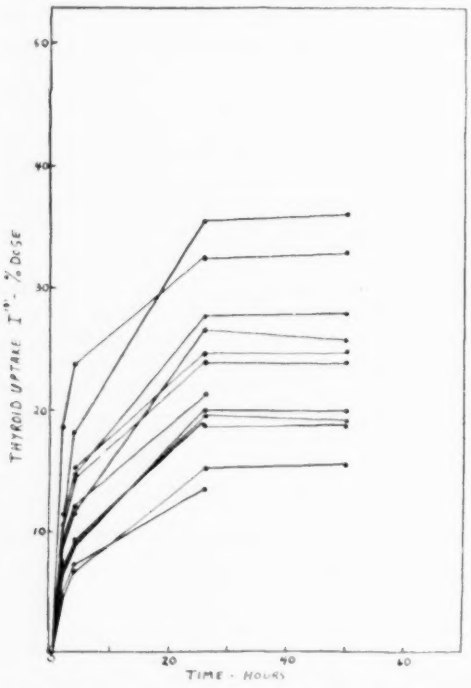

FIG. 1. THYROID UPTAKE OF RADIO-IODINE BY CONTROL SUBJECTS

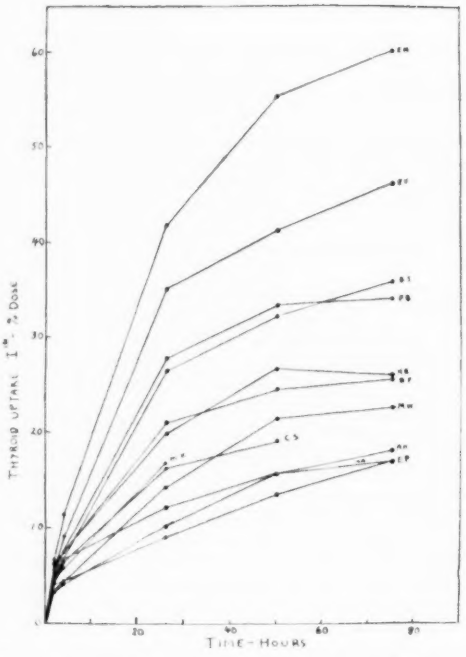

FIG. 2. THYROID UPTAKE OF RADIO-IODINE BY SUBJECTS WITH RENAL DISEASE

I^{127}. As a higher percentage of filtered I^{131} was reabsorbed by the tubules, consequently much more iodide I^{127} was also being reabsorbed by the sick subjects.

It was noted in the few subjects in which it was done that the percentage tubular reabsorption of filtered sodium and chloride was if anything somewhat less in the patients with kidney disease. This is in marked contrast to their handling of the other halogen iodide. These figures are set forth in Table V.

(*B*) *Results on the thyroid uptake of radio-iodine in kidney disease*

Most of the control subjects had taken up their maximum amount of I^{131} into their thyroids by 26 hours after the dose. This is illustrated in Figure 1. In a few cases there was a further small increase during the following 24 hours. This never amounted to more than 1.4% of the dose. The results obtained in 12 patients with kidney diseases, shown in Figure 2, were markedly different. It

TABLE VI

*The thyroid clearance and thyroid uptake of I^{131}; the fasting plasma I^{127} level and thyroid uptake of I^{127}.**

Subjects	Thyroid clearance		Thyroid uptake I^{131}		Plasma I^{127}		Thyroid uptake I^{127}	
	no.	*ml./min.*	*no.*	*%/hour*	*no.*	*μgm./100 ml.*	*no.*	*μgm./hr.*
Controls	12	12.1±2.5	11	4.2±0.6	7	0.17±.005	7	1.08±0.23
Renal disease	11	4.3±1.5	10	1.8±0.2	6	2.20±0.50	6	2.9 ±0.80
P values	<.01		<.01		<.01		<.05	

* Mean values ± standard error.

will be seen that while at 26 hours after the dose the thyroid uptake was not greatly dissimilar from the controls, it was in all cases followed by further increases. One case (E. M.) with chronic nephritis showed a rather odd curve for I^{131} thyroid uptake with a maximum uptake reached at 75 hours of some 60% of the dose. This patient was clinically euthyroid and had protein bound plasma iodine level within normal limits. Values derived from this patient were not included in the series.

Corresponding with the flattened thyroid uptake curves it will be noted in the patients with renal disease that the mean rate of thyroid clearance of plasma of I^{131} and also the rate of uptake of I^{131} by the thyroid are considerably less than these same rates in control subjects. These values are shown in Table VI. On the basis of these figures as well as from the extra-renal disposal rates reported above one might conclude that the thyroids of persons with renal disorders were hypofunctioning. However, as is also shown in Table VI, when the actual quantities of stable iodide I^{127} entering the gland are considered, the mean rate of uptake of I^{127} by seven control subjects was found to be 1.08 μgm. per hour under fasting conditions while in six persons with renal dysfunction it was found to be 2.9 μgm. per hour, a highly significant difference. It would thus appear that the thyroid gland in kidney disease is not hypoactive and actually more iodine per unit time is entering the gland under fasting conditions. As all of the subjects were clinically euthyroid and none had the elevated levels of protein bound plasma iodine characteristic of hyperthyroidism, it would appear that despite the enhanced uptake of iodine into the thyroid, there was no increased production of hormone by the gland.

DISCUSSION

Since there was much evidence of kidney parenchyma damage including elevated plasma creatinine levels in the subjects with renal disease it is not surprising that decreased renal clearance rates for radio-iodine and elevated plasma levels at 26 hours were found.

It has been shown by Riggs that iodide clearance rates in normal dogs can be greatly decreased by low sodium chloride intake (15). Of the subjects with kidney disease here studied, seven were on low salt intake, but three were on normal diets and one on normal diet plus 2 gm. extra salt per day and these four show an average radio-iodide clearance of 4.6 ml./min., a value which is somewhat below that of the whole group. Thus although a low sodium chloride intake may bring about a decreased clearance of iodide from the blood, the main cause of the decreased rate in the subjects with renal disease was not this factor but more probably the glomerular and tubular pathology.

The glomerular filtration rate as measured by the endogenous creatinine clearance was found to be decreased. This resulted in a decreased filtration of radio-iodide and consequently a considerable retention of the isotope due to the glomerular damage. However, the retention of I^{131} was not entirely due to poor filtration for there was also increased tubular reabsorption of the filtered radio-iodide. When the quantities of stable iodide involved were calculated, it was shown that despite depressed glomerular function actually more iodine was being filtered per hour than by normal subjects and of the amount filtered, a greater portion was being reabsorbed by the tubules.

A dissimilarity of tubular function in respect to sodium and chloride on the one hand and iodide on the other has appeared in the present study.

The percentage of resorption of filtered iodide has been estimated to be 73% by Smith (16). Results in the present study using physiological amounts of iodide labelled with the I^{131} agree well with this figure. It is remarkable that chloride which is chemically similar to iodide is resorbed by the tubules in percentage amounts similar to those noted for sodium, namely 98–99% of the tubular load. It has recently been shown by Platt (17) that although patients with renal failure filter less sodium than normal, they resorb a smaller percentage of the tubular load. This was noted also for sodium and chloride in two patients in whom it was examined in the present series. It is therefore interesting that in patients with renal failure there is an increase in the tubular reabsorption of both I^{131} and I^{127} but a fall in the amount of filtered sodium and chloride reabsorbed.

Platt (17) found the degree of reduction in tubular reabsorption of sodium by patients with renal disease was directly related to the amount of decrease in glomerular filtration, thus maintaining a normal plasma level of sodium. In the present series neither a correlation between filtration rate and the degree of iodide resorption nor a maintenance of normal plasma iodide levels was noted.

Since urinary excretion of I^{131} is much lower than in the control subjects, erroneous interpretations as to thyroid function will be made from urinary excretion values for radio-iodine in kidney disease; likewise misleading results will be obtained from any figures derived originally from urinary excretion data such as the "extra-renal disposal rate" and the "thyroid accumulation rate" of Keating and associates (7).

The observation that the thyroids of patients with kidney disease continued to take up radio-iodine after 26 hours, in contrast to those of normal individuals, is most likely due to the fact that such thyroids are presented with higher plasma levels for longer periods of time. In the patients with kidney disease who, as noted, were clinically euthyroid and had normal levels of protein bound iodine in the plasma, there was a significant decrease in the rate of clearance of radio-iodine from the plasma by the gland. This could be due to either decreased activity of the gland or to dilution of the isotopic iodine with the high plasma levels of stable iodine or to both. In six patients in whom it was indirectly measured, the fasting plasma I^{127} level was on the average over 10 times higher than in the control subjects, therefore considerable dilution of the I^{131} by the stable isotope occurred, thus explaining in part the decreased uptake of I^{131}. As regards the possibility of a decreased avidity for iodine of the thyroid glands of subjects with renal disease, on calculating the actual hourly uptake of I^{127} by the glands, it was shown that the amount of iodide entering the gland per hour was actually higher than in the control subjects. As the patients were clinically euthyroid with normal levels of protein bound plasma iodine, it would seem that hormone formation was not increased. The increased entry of iodide molecules cannot be explained on the basis of an increased accumulation, for the capacity of the gland to hold iodide cannot be infinite. It must therefore be assumed that any increased uptake of inorganic iodine is balanced by an increased loss. This implies an increased rate of exchange between the inorganic iodine of the gland and of the plasma, the precise condition which would be expected in the presence of higher iodide concentrations in two tissues in equilibrium with each other. That there exists a dynamic equilibrium between the iodide of plasma and that of thyroid over a wide range of plasma iodide concentrations has been shown by Vanderlaan and Vanderlaan (18).

Despite the diminished clearance of I^{131} from the plasma and the decreased rate of accumulation of I^{131} by the thyroid, it was noted that the 26 hour thyroid uptake, though lower than similar determinations on control subjects, was not greatly so. It would seem that two opposing factors are at work—a dilution factor due to elevated stable iodine plasma levels which lowers the amount of the radioactive isotope entering the gland, and a retention factor which presents the thyroid with higher levels of I^{131} for a longer time.

In 11 patients the 26 hour uptake of radio-iodide was within normal limits except in one elderly man where it was quite low. It has been noted, however, by Perlmutter and Riggs (19) that low uptakes of radio-iodide are common in the aged. It would seem therefore that where the question of thyroid assessment comes up in subjects with renal disease and an isolated procedure is used, the 24–26 hour *in vivo* measurement of thyroid uptake of I^{131} gives reasonably accurate

information. This is in marked contrast to urinary excretion data or thyroid clearance or accumulation rates of I^{131} which, if carried out on patients with renal failure, can give a quite false picture of thyroid function.

SUMMARY

The urinary excretion and thyroid uptake of radioactive and stable iodide under fasting conditions were studied in a group of patients with renal disease and compared with similar values in a group of control subjects.

It was found that there was a low rate of excretion and a depressed renal clearance of iodine isotopes but in the patients with renal disease a greater proportion of the tubular load was reabsorbed. This was in contrast to the tubular handling of another halogen ion, chloride.

The rate of accumulation of radio-iodide by the thyroid gland was depressed as was also the "extra-renal disposal rate" of iodine calculated from urinary excretion data. Low values were also found for plasma clearance of radio-iodide by the thyroid gland. This was ascribed in part to a dilution of the radioactive isotope by retained stable isotope of dietary origin. When the amounts of stable iodide (I^{127}) involved were computed it was found that actually more iodide was entering the thyroid per unit time than was the case in the control subjects. As the subjects were euthyroid and had normal levels of protein bound iodine in the plasma it was concluded that the increased uptake of iodide by the gland did not lead to an increased production of thyroid hormone.

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RELATIONSHIP OF ELECTRICALLY INDUCED PAIN TO THE AMPERAGE AND THE WATTAGE OF SHOCK STIMULI

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INTRODUCTION

The present paper deals with the development of an apparatus for delivering controlled electric stimuli, and with an attempt to discover which physical aspect of 60 cycle alternating current (voltage, amperage, or wattage) is most closely related to discrimination of shock stimuli in psychological experiments.[1] Shock, of course, has been the most widely used, and the most *convenient* type of noxious stimulus for many psychological studies. However, "convenient" should be italicized for, while shock is usually rather simple to apply, accurate control of the most effective physical aspect of this stimulus is difficult.

A survey of the literature indicates that control of voltage alone in psychological studies is quite unsatisfactory. This is frequently the case even when considerable physical resistance is added to the circuit in series with the biological materials.

Problems relating to the use of electric shock stimuli were considered in a round-table discussion of the American Psychological Association (2, 3). Although there was no close agreement among members, the general opinion seemed to be that amperage was the physical aspect of shock which should be controlled. Muenzinger and Walz (4) developed a method for controlling *D.C.* current (to a great extent) despite variations in skin resistance. This technic has proved quite satisfactory for a number of purposes. However, the few psychophysical experiments that have been performed with control of amperage have yielded disparate results. Thus, Dalziel and Lagen (5) found that the integumental threshold for 60 cycle alternating current was 1.1 milliamperes (ma.), while Forbes and Bernstein (6) found great fluctuations. In a study using a modified method of limits while controlling wattage, Gilmer (7) found a mean absolute threshold at 0.3 ma. at a frequency of 64 cycles per second, and a mean threshold value of 3.0 ma. at a frequency of 12,500 cycles. It is known, of course, that differences in electrode materials and differences in their size, shape and location may introduce artifacts; however, it would seem that the discrepancies have been so great that they cannot be accounted for on the basis of electrode error. It is evident, therefore, that the possibility must be considered that power may be the most relevant measure of electric shock stimuli in psychological studies. Such a conclusion was reached by Forbes and Bernstein (6) who stated that "neither voltage nor current alone is a satisfactory index of the sensory effects of shock. The results *do* suggest that power *may* be such an index."

Quite uniform and stable power thresholds were obtained by Gilmer in the study mentioned above (7). He used a method of limits in which a milliwatt (mw.) scale was employed for determining limens at nine frequencies that ranged from 64 to 12,500 cycles per second. Thresholds were found to be almost identical for his four subjects, being 30.88 mw. at 64 cycles and 392 mw. at 12,500 cycles.

In support of the hypothesis that power is the most significant physical aspect of shock, it might be noted that several of the devices used for controlling amperage also reduced variations in power. Certainly Muenzinger and Walz (4) reduced the variation in power as they reduced the error in amperage. Also, it might be pointed out that the introduction of complemental resistance into the stimulating circuit in physiological experiments seems to reduce fluctuations in stimulus power. Thus, Jasper (8), in adding 15,000 ohms in series with the subject during studies on chronaxie, was

[1] Ramifications of the obvious fact that the value or function that shock acquires is different for different individuals will not be considered in this paper; *cf.* Tompkins (1).

in effect exerting control of the energy of the stimulating circuit.

There are indications, also, that power has been partially controlled in conditioning studies. For instance, in studies on dogs, Kellogg (9) maintained a voltage that was just sufficient to force a flexion reflex of four inches. Kellogg, Pronko, and Headlee also used this method for investigations of drugs (10) wherein they believed it necessary to control the unconditioned response according to a behavioral index of the effectiveness of the shock. Later Spooner and Kellogg (11) extended this type of shock control, on the basis of a reaction index, to the conditioning of the finger withdrawal in human subjects. Inspection of Kellogg's (9) voltage curves suggests that the uniformity of the unconditioned response was achieved through at least partial control of the power of stimuli. This is indicated by the gradual decline in the voltage necessary to elicit the unconditioned response as the experiment progressed. Since it may be assumed that the resistance of the biologic circuit declined progressively after repeated shock stimuli, it is apparent that the voltage required to deliver a constant power would also be reduced concomitantly.

The control of shock stimuli in terms of the physiological reaction which they produce has been employed in other areas of conditioning and in neuropharmacological investigation (Wikler [12]). However, it seems to be somewhat inadequate for human studies. One of the reasons is that the frames of reference are different. Extrapolation from results obtained in studies where a reaction was forced by shock, to results obtained in human discrimination of the intensities of stimuli, would seem unwarranted. It would be desirable, therefore, to discover the relative effectiveness of amperage and wattage in representative experimental situations; *e.g.*, in purely physiological studies, in conditioning studies, in discrimination studies and in any other situations displaying unique characteristics. It may be that the effective electrical index in one will be found to be the effective index in all others; this remains to be tested.

Our own pilot work was begun with a modified method of single stimuli using a voltage scale. Standard stimuli were applied one hour previous to the administration of six test series of 11 stimuli each. The subject reported verbally his judgment of whether the variable shocks were "stronger" or "weaker" than the previously given standard. Both delivered voltage and amperage were recorded for each stimulation. Results, computed separately on the basis of delivered voltage, amperage and power, showed that voltage was the least significant characteristic; also these computations gave strong indications, but did not prove, that power was the most important physical characteristic affecting judgments.

This work showed one thing quite conclusively. If experiments of any precision were to be undertaken using shock stimuli, a method must be developed for accurately predicting the voltage necessary for producing desired amperages or wattages once the skin resistance was known. A direct application of Ohm's law could not be relied upon in this situation since it is known that capacitance is a factor of some magnitude which must be taken into account in studies on biological circuits (Curtis [13]), especially when skin impedance is relatively high. Therefore, for present purposes it was necessary to devise an apparatus which could be used to deliver shock stimuli of known wattage or amperage, and to construct empirical power curves for the purpose of pre-setting the stimulator for desired shock intensities. After this was accomplished, a procedure was developed to determine the relationship of induced pain to the wattage and amperage of electric shock stimuli.

METHODS

Apparatus. The biologic resistance of the subject before and after stimulation was measured by use of a General Radio *A.C.* bridge (Type 650 A). In making such measurements the capacitance of the subject was balanced out by adding the required capacitance to the opposite arm of the Wheatstone bridge. A cathode ray oscilloscope was used to determine the state of balance in the bridge circuit.

The circuit of the 60 cycle *A.C.* stimulating apparatus is illustrated in Figure 1. Essentially, this consists of two parts: (*1*) a stimulating circuit which permits recording of impressed voltage and actually delivered amperage, and (*2*) a trigger and relay system which limits the duration of the stimulus to exactly 0.1 second and prevents accidental repetitive shocking of the subject during a 30-second period after each stimulation.

The stimulating circuit is composed of a line source variac and transformer which permits delivery of 0–600 volts *A.C.* to the subject through an external resistance of 10,000 ohms. The latter serves to minimize the effects of variations in the subject's impedance during passage

of the stimulating current. Root-mean-square amperage of the stimulating current actually delivered to the subject is measured by a separate circuit (insert, Figure 1). Flow of current in this circuit is induced by that passing through the subject during stimulation. The *A.C.* induced current is converted by a rectifier into *D.C.* surges which charge a series of condensors. The resulting voltage drop across one of the condensors, as measured by a standard *D.C.* vacuum tube voltmeter, is therefore proportional to the *A.C.* current passing through the subject.

The trigger and relay system limits the stimulating current to a duration of 0.1 second by a circuit consisting of Relay #1, resistor R_1, condensor C_1, and thyratron tube *2D21* and a power source *P*. As long as contact *B* is closed, current flows through this circuit, Relay #1 remains activated and contacts *A* and *D* remain open. When contact *B* is opened, this circuit is broken, thereby permitting contacts *A* and *D* to close and the stimulating current to pass through the subject. The opening and closing of contact *B* is almost instantaneous (see below). Hence, the circuit is restored almost immediately after stimulation is begun. However, contact *A* remains closed until Relay #1 is reactivated. This interval (0.1 second) is determined by the characteristics of thyratron tube *2D21*, R_1, and C_1, since immediately after the opening of contact *B*, condensor C_1 discharges and when its potential drops to about zero, the thyratron tube is fired and Relay #1 is reactivated, thereby opening contacts *A* and *D*. Prevention of accidental repetitive shocking of the subject for a period of 30 seconds is insured by the circuit consisting of switch S_1, condensor C_2, resistor R_2, and Relay #2. When switch S_1 is closed (by the subject or the experimenter), condensor C_2 discharges, thereby activating Relay #2 and opening contact *B* for a very brief period (approximately 10 msc.). The values of C_2 and R_2 are such that 30 seconds are required for recharging C_2 to a voltage which is high enough again to activate Relay #2.

Subjects. All subjects used in this investigation were male prisoner patients with previous histories of drug addiction ("post-addicts") who had not received drugs for several months prior to the present study, and who

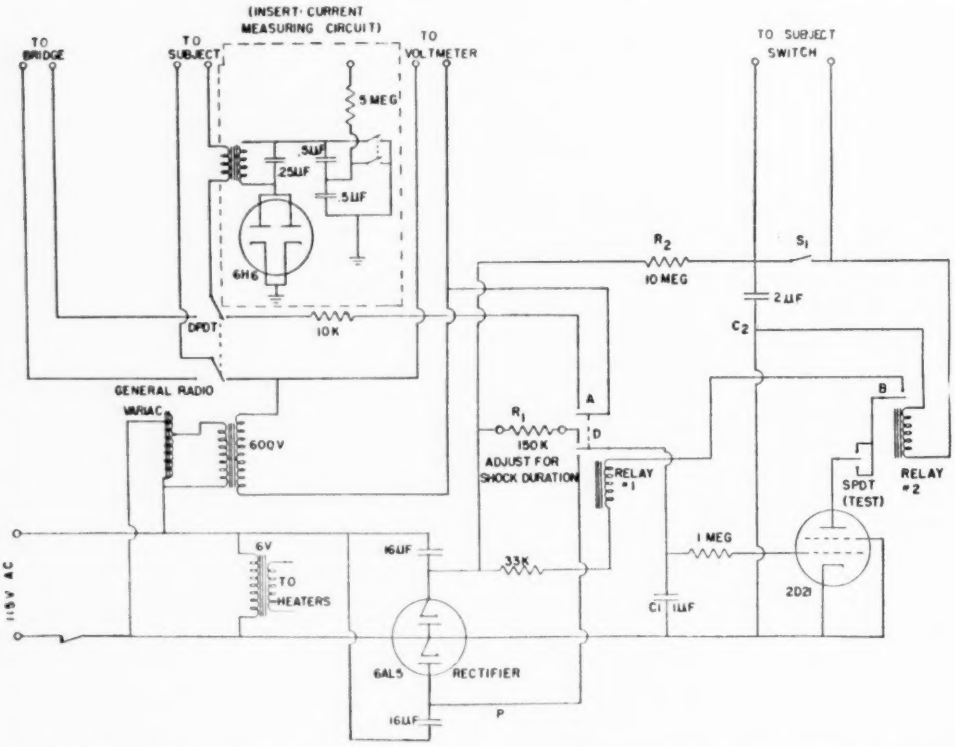

FIG. 1. ALTERNATING CURRENT SHOCKING APPARATUS: 60 CYCLE STIMULATING CIRCUIT, STIMULUS DURATION CONTROL AND SAFETY DEVICES

For explanation see text.

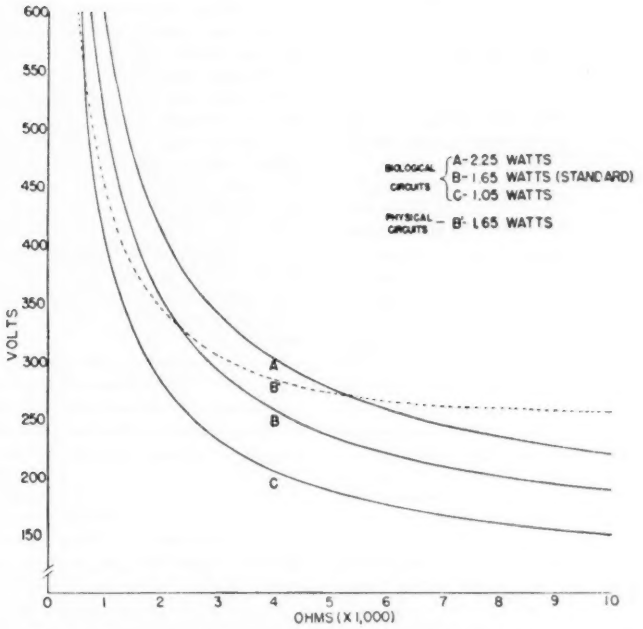

FIG. 2. RELATIONSHIP BETWEEN VOLTAGE AND RESISTANCE FOR THE STANDARD POWER OF 1.65 WATTS IN A PURELY PHYSICAL CIRCUIT (B'), AND SIMILAR RELATIONSHIPS FOR SEVERAL BIOLOGICAL CIRCUITS
For explanation see text.

volunteered to serve as subjects for these experiments. Forty individuals were used in experiments which were made for the purpose of constructing empirical and rational power curves and 12 others served in experiments which were designed to determine whether electrically induced pain is related more closely to the amperage or the wattage of electric shock stimuli.

Procedure. A. Construction of empirical power curves. The following rather simple method was used for this purpose: (*1*) stimulating the experimenter and other "willing" subjects at progressively increasing intensities to a power level that was definitely painful; (*2*) selecting a group of subjects whose skin resistance would cover the range to be expected; (*3*) stimulating these subjects with powers that varied randomly, from those which were quite low to those which were considerably higher than the level which was painful to the experimenters; (*4*) plotting the delivered powers, from calculations of $P = I^2R$, as functions of skin resistance on the abscissa and voltage on the ordinate; (*5*) sketching a power curve covering all resistances, that was equal to the power reported as painful by the experimenters; (*6*) sketching two end power curves, one lower and one higher than the standard which were equidistant in power from the latter, and which could be distinguished from it in approximately 100 per cent of the trials; (*7*) interpolating, according to the calculated powers, four steps between each end point and the standard, thereby providing a scale of nine powers. (Only three curves are presented in Figure 2; these are for the standard and the two end powers.)

It was found that biological resistance, not including complemental resistance, ranged from approximately 700 to 10,000 ohms. For the reasons stated above, a complemental resistance of 10,000 ohms was added in series with the subject in the stimulating circuit. It will be noted, however, that biological resistance, or its equivalent, and not total resistance has been plotted in Figure 2. The addition of 10,000 ohms of complemental resistance inverts the power curves from the position they would occupy were power calculated on the basis of the biological resistance alone as long as the biological resistance is less than the complemental resistance. (Simple calculations on the basis of Ohm's law will show that when the biological resistance is greater than the complemental resistance, the voltage which is necessary to deliver any given power to the subject increases concomitantly with increase in biological resistance.) Necessary voltage, on the ordinate, for delivering desired powers as these biological resistances, ranged from 150 to 600 volts. The power scale finally decided upon was built around a standard of 1.65 watts. These powers were approximately 1.05,

1.20, 1.35, 1.65 (standard), 1.80, 1.95, 2.10, and 2.25 watts.

In employing the charts for the purpose of delivering shock stimuli at any desired power, the appropriate power curve was identified and the necessary shocking voltage was determined for the particular skin resistance (complemental plus subject's resistance) before each stimulation.

B. Relationship of induced pain to characteristics of shock stimuli. The method for determining the relative effectiveness of amperage and power was rather simple after the apparatus and the empirical power curves were developed, and after it had been shown that skin resistance could be markedly reduced by scrubbing the fingers with saline paste. The 12 post-addict subjects were all given the same treatment and no medication was administered. After each subject was shown into the quiet, air-conditioned experimental room, the electrodes were applied and held in place by a ¾ inch rubber band. The electrodes consisted of two stainless steel plates measuring approximately 3 × 5 cm. which fitted the contour of the dorsal and palmar surfaces of the fingers. Each plate was connected to the stimulating apparatus by means of insulated wire leads which were fixed to binding posts in the center of the electrodes. They were coated with saline paste but were applied *without* scrubbing the fingers. Skin resistance was then obtained after balancing the capacitance. Under these conditions most individuals' skin resistance was found to be relatively high.

Six shocks of progressively increasing power were then delivered terminating with two of the standard stimuli of approximately 1.65 watts.[2] To give the subject practice in comparing intensities, a complete series of nine shocks was administered in predetermined random order at approximately 45-second intervals. The subject responded to each shock by reporting verbally whether it was stronger or weaker than the previously given standards. (No equal judgments were allowed.) On completing this practice series, the subject was given four more stimuli of standard strength, preceded by the following information: he was told to remember the strength of these standards since, after a one-hour interval, his judgment would be required on several more series.[3] The subject was then returned to the waiting room.

The subject returned to the experimental room after the one-hour interval, and the electrodes were re-applied. However, at this sitting, the subject's fingers were prepared somewhat differently. The fingers were cleaned with acetone and both surfaces were scrubbed thoroughly with a toothbrush coated with electrode paste. The lateral surfaces were wiped with acetone, and the electrodes applied with a uniformly thin covering of saline paste. Two test series, similar to the practice series except for differing stimulus order, were then administered. (The first stimulus for the first series was at a considerably *lower* power than the standard.) Prior to each of the series the subject was told to judge each shock as to whether it was stronger or weaker than the previously given standards, and not to compare them with each other.

The manipulation of skin resistance provided the pertinent data. Applying the electrodes without scrubbing the fingers previous to the practice series and administration of the four standards, resulted in standard shocks being given at relatively high and constant biological resistances. Thus, each individual received shocks of standard amperage and standard wattage. However, when the subject returned for the test series, the scrubbing procedure reduced the skin resistance markedly. The test series were power series; therefore, regardless of biological resistance, approximately the same powers were delivered that had been given during the practice series. But, due to the decrease in biological resistance, amperage, in contrast to power, was greatly increased over that given during application of the standards.

Thus, upon beginning the test series, the subjects had equal opportunity to judge on the basis of either amperage or power, since they had received standards of each, central tendencies being equal. As indicated, in order to make the first judgment decisive, a power much lower than the standard was administered as the first test stimulus. The power for this first stimulus was much *lower* than the standard, whereas, for the majority of subjects, amperage was much higher than the standard because their biologic impedance had been reduced greatly by the skin-scrubbing procedure. Hence, if the subjects judged the intensity of the stimulus on the basis of its power, they would respond "weaker," whereas, if judgments were made on the basis of amperage the response would be "stronger."

Treatment of data. Errors in discrimination were compared. Those that were made when calculations were based on amperage were compared with those made when calculations were based on wattage. The first comparison was between errors in judgment on the first stimulation of the first series; it was so clear-cut that no statistical tests of significance were required.

[2] We found frequently in our exploratory work that the first and sometimes the second stimulation produced greater power than predicted; thus during the initial stimulation for all individuals, the delivered power of each stimulus, including the first two standards, was calculated immediately after stimulation, using the formula I^2R. This procedure provided a check on the standard power, as it was occasionally necessary to shift the voltage upward or downward to obtain the desired power. Also, this procedure obviated excessive stimulation.

[3] This method of administering the standards an hour previous to the experimental series was employed to parallel a subsequent discrimination study (14). In that study it was necessary to determine whether morphine impaired the ability to discriminate different intensities of painful stimuli; therefore, all the pre-test standards were given under non-drug conditions and the one hour interval provided time for the administration and action of morphine.

However, as one would expect, there were errors on both indices when a comparison of total errors was made for Series 1 and Series 2. These were evaluated by using the difference method of the *t* test (Edwards [15]).

RESULTS

The functioning of the apparatus was satisfactory for our purposes. The mean of the delivered preparatory standards for 30 subjects was 1.66 watts, and the mean of the delivered series standards was 1.64 watts (predicted: 1.65 watts). The standard deviation of the differences between the preparatory and series standards for this group was 0.039 watts.

It was found, with one exception, that the voltage necessary to obtain a particular power in a biological circuit of a certain measured resistance does not correspond with the voltage calculated by means of Ohm's law. In Figure 2 are shown the relationships between E and R in a purely physical circuit that includes only resistance (curve B'), and three different apparent powers in biological circuits (curves A, B, and C).[4] The biological circuit included the subject's pre-stimulation resistance as well as unknown capacitance and inductance. It will be noted that for resistances that are considerably higher or lower than 2,300 ohms, the biological E–R curve for the standard power diverges somewhat from the equivalent curve for physical power. In all probability this is because the effective E for a biological circuit of unknown capacitance and inductance is a function of these variables and therefore is apt to be different from the pre-set E which was estimated from calibrations on a purely physical circuit. Although changes in impedance following shock could not be measured, it was found that capacitance increases as biological resistance increases, being of the order of .05 microfarad at 10,000 ohms. It will be noted that the greatest divergence of the "physical" and "biological" curves occurred in the region at which pre-stimulation capacitance was greatest (Figure 2).

As would be expected, the E to I relationships for the biological circuits also did not conform to those which would be anticipated on the basis of Ohm's law in a physical circuit. The measured I appeared to be largely independent of the pre-stimulation resistance, and was almost entirely a function of E. Therefore, as R decreased, both E and I were increased for any particular delivered power.

The first treatment of the data of the experiments on the relationship of pain to the characteristics of shock stimuli was a comparison of errors for the first test stimulations. Scrubbing the fingers with saline paste before applying the electrodes for the test series resulted in a drop of skin resistance from a mean of 5,567 ohms for the preparatory standards to a mean of 1,758 ohms for the first test stimulation. Mean power and mean current for these two conditions changed from 1.68 to 0.87 watts, and from 17.9 to 22.8 ma., respectively. Nine of the subjects received greater amperages on the first test stimulation than on the standard, while in all cases power was much less than the standard. All subjects who had a clear-cut choice responded in favor of power, *i.e.*, 100 per cent gave the response "weaker." This finding is all the more significant since, in many cases, the difference in amperage was much greater than indicated.

Table I shows the significance of the differences between total errors in judgment when all responses were evaluated in comparison with de-

TABLE I

Significance for differences between the mean errors in judgment on amperage as opposed to those on wattage

	Mean errors		Mean difference	Sigma mean difference	t	P*
	Power	Amperage				
Series 1	2.08	4.42	2.34	0.742	3.15	<1%
Series 2	2.00	4.67	2.67	0.689	3.88	<1%

* The P column indicates the percentage of such results that would be expected to occur by chance.

[4] In Figure 2, curve B' represents the relationships between E and R for one given power (standard) as calculated from the equation $E = \sqrt{PR}$ which is another form of Ohm's law for a physical circuit. Curves A, B, and C are rational curves for biological circuits derived from the equation $E = \sqrt{\frac{10\,P\,(R + K)}{d}}$, in which $d = \frac{10\,R}{K} - \sqrt{\frac{R}{R + K}}$. These curves were found to correspond quite closely to the actual plots of *observed* E and R for any given power as calculated by the equation $P = I^2R$. Possible reasons for the differences in $E - R$ relationships in biological and physical circuits are discussed in the text. The use of the rational equation has the advantage that P can be calculated in terms of E and R alone, without the use of I. However, curves identical with these would not be expected if the stimulator components were changed since the internal resistance and internal conductance would vary from instrument to instrument.

livered amperage or calculated power. The first comparison is for Series 1. Only one of the subjects made more errors when calculations were based on power than when they were based on amperage (this subject's judgments were quite erratic). The *t* between power and amperage was 3.15. This probability of chance occurrence (less than 1 per cent), which is statistically very significant, would have been even less if a greater number of subjects had been tested or if more control of skin resistance had been obtained.[5]

Table I also presents a similar comparison for Series 2. The errors on amperage in relation to those on power were even more marked than for Series 1; the *t* between errors was 3.88, giving a probability of chance occurrence of less than 1 per cent. The significance of this *t* for Series 2 may be somewhat attenuated, however, since central tendency of judgments may have begun to operate.

DISCUSSION

The apparatus functioned satisfactorily in our experimental situation, and the errors between predicted and delivered power were comparatively small. Nevertheless, if much smaller step intervals of power were used, or if an experimental design necessitated great precision, further refinement in control would be needed. However, it would appear that the accuracy which was attained would be sufficient for the majority of psychological studies. Variability of several hundredths of a watt very probably would not be reflected in the outcome of conditioning work. Certainly for experiments in discrimination, and for other studies that we have underway in which short term disruption of behavior is introduced by shock, our control of power is far superior to control of voltage, or of amperage as previously used.[6]

Aside from comparatively small errors in predicting delivered power, there are several disadvantages in the present form of the apparatus. The complexity of design requires the services of either a physicist or a person of considerable electronic inclination. This complexity also makes it an impractical instrument for the average psychological or physiological laboratory in which severe limits are frequently placed upon expenditures. However, further work will probably result in a simplified method for controlling power.

As indicated by Curtis (13), it was maintained for some time that a completely new set of laws other than those describing physical circuits would have to be developed for assaying biological materials. However, as he points out, the apparent discrepancies may be quite easily due to the complexity of biological circuits, and to the great difficulty of compensating for, of controlling, or even of measuring changes in the material under study.[7]

As indicated, one of the sources of error may possibly be that of shock-induced changes in capacitance or in the other characteristics of impedance. In a circuit that includes resistance, capacitance, and inductance, power is expressed by the equation $P = I^2Z$. Z, the impedance of the circuit, is a function of resistance, capacitance, and inductance, the value of which can be determined only if the values of these variables and their series or parallel relationships are known. Obviously, except for the subject's pre-stimulation resistance, such factors are unknown in the biological circuits obtained in the present experiment. Hence, in this paper the term "power" has been used to indicate the apparent power given by the equation $P = I^2R$. Although such a power index may not correspond in all instances with the true power, it has considerable utility in that its

[5] Several factors were operating against an outcome in favor of power: (*1*) as shown in a separate study (**14**), if the subjects were "anxious" they would tend to report stimuli as being "stronger"; (*2*) power was occasionally greater on first stimulations than predicted; (*3*) if the scrubbing procedure sensitized the skin there would be a tendency to report "stronger" more frequently.

[6] Although extrapolation may not be warranted from our results on verbal report to the effective stimulus in electroshock convulsions, investigations of controlled power in such situations might result in more adequate control of induced seizures. Arieff's paper (16) indicates some of the difficulties in this area.

[7] Over 40 years ago, it was pointed out that in physiological studies, electric stimulators should be calibrated in terms of a particular physiological response (Martin, E. G., A quantitative study of faradic stimulation. I. The variable factors involved; and V. The influence of tissue resistance and of kathode surface on stimulating effectiveness. Am. J. Physiol., 1908, **22**, 61; and 1910, **27**, 226). This investigator demonstrated that the contractions of frog's gastrocnemius muscle correlated fairly well with the amperage of make or break faradic shock stimuli. He did not investigate the relationships between such responses and the wattage of electric shock stimuli.

use makes possible the control of stimulation with respect to a physical measurement which, as has been shown, correlates highly with estimation of pain intensities. Actually, as mentioned, for resistances of about 2,300 ohms, "apparent power" corresponded quite closely with calculations of power based on Ohm's law in a purely physical circuit which included only resistance. In addition, the data show that power (I^2R) may be predicted in a biological circuit with a comparatively small degree of error provided the pre-stimulation resistance of the subject is known and the initial impedance of the stimulating source is kept constant.

The main purpose of the experiment was to determine which characteristic of electric shock stimuli, amperage or power, was more closely related to the subject's report of the intensity of electrically induced pains. The two types of analysis of the data were in very close agreement. The data prove conclusively that power is the more important physical variable in determining verbal reports of the intensities of shock stimuli. In several studies mentioned in the introduction and in our own pilot work, evidence was adduced showing that voltage is a less significant variable than amperage. Therefore, we are led to the conclusion that of the three physical variables of shock stimuli, wattage is the one that should be controlled. When this is done, electric shock stimuli may prove to be not only a convenient stimulus for use in psychological studies on pain, but also an accurate one.

It should be pointed out that in our experiment the delivered powers of electric shock stimuli were nearly equivalent to delivered energies, since the duration of the stimulus was always 0.1 second. In this connection it is pertinent to note that the delivered energy of radiant heat stimuli has also proved to be useful in studies on pain (Hardy, Wolff, and Goodell [17]). This suggests the possibility that with any given technic for inducing pain, the relationship between pain intensity and the physical aspect of the stimulus can be described most accurately when the latter is expressed in terms of energy appropriate to its source—electrical, thermal, mechanical, photic, sonic, etc.

SUMMARY

1. An apparatus has been described that could be modified for controlling either the delivered wattage or the delivered amperage of electrical stimuli of fixed duration, and which provided a safety device to avoid accidental repetitive shocking.

2. With one exception, the data obtained indicated that the power developed by a particular voltage in a *biological* circuit of a certain measured resistance does not correspond with the power developed at the same *physical* resistance by the same voltage. This suggests that a biological circuit is characterized not only by resistance, but by unknown capacitance and inductance. Therefore, delivered wattages calculated on the basis of $P = I^2R$, where R is merely the pre-stimulation biological resistance, are only "apparent" powers. However, under the conditions of our experiments, such "apparent power" corresponded quite closely to I^2R values calculated on the basis of an equivalent physical circuit.

3. Post-addict subjects judged the intensities of electric shocks delivered by the apparatus. The correspondence of the subject's verbal reports with the amperage and the wattage of stimuli was measured separately, and the differences were statistically evaluated. After a practice series which was followed by the application of four stimuli of standard amperage and wattage, resistances were reduced by scrubbing with saline paste the surfaces to which the electrodes were applied. Two consecutive power series of nine stimuli each were then administered. In each of these test series, one stimulus was of the standard power and four were higher and four were lower powers than the standard. Due to the decrease in resistance following the scrubbing procedure, the amperages of the test stimuli were considerably higher than those of the practice period. In response to each stimulus, the subject reported whether it was "stronger" or "weaker" than the previously administered standard.

4. It was shown quite conclusively that apparent delivered wattage correlates more highly than amperage with estimation of the intensities of electric shock stimuli.

5. The application of these findings to studies on pain and to other problems is discussed.

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EFFECTS OF ANXIETY AND MORPHINE ON DISCRIMINATION OF INTENSITIES OF PAINFUL STIMULI

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INTRODUCTION

The testing of analgesics, especially morphine, has yielded a great deal of conflicting evidence concerning the effects of drugs on pain perception. The recent reviews by Wikler (1) and Edwards (2), and the monograph by Wolff and Wolf (3), cover thoroughly the studies that have attempted to measure changes in the pain perception threshold. Although many investigators have reported rises in the pain perception threshold of some of their subjects following the administration of morphine, the majority have obtained variable results. Wolff, Hardy, and Goodell (4) found consistent rises following the administration of morphine under certain conditions. Using the same technique, Andrews (5), Isbell (6), Denton and Beecher (7), and Chapman and Jones (8) found, following morphine, that the pain perception threshold might be elevated, lowered or unchanged. Similar results were obtained by Isbell and Frank (9) in studies on the effect of analgesics on tooth pain perception thresholds.

It would seem that the discrepancies were not due to faulty apparatus or lack of objectivity in handling the data. Rather, the reasons appear to lie in conditions that were not held constant or that were beyond the control of the experimenters. One such variable that has been mentioned in several studies as possibly contributing to unpredictability of results is the "emotional" status of the subject at the time the experiment is performed. Included would be such factors as response of the subject to the experimental room, to the complex apparatus, to the attitude of the experimenter, and to the expected painful stimuli. The uncontrolled variable in the subject's behavior would then seem to comprise that class of responses which is termed anticipatory, *i.e.*, especially affectively toned responses that are anticipatory of pain. This, of course, is one type of fear or "anxiety."

Isbell and Frank (9) also studied the effect of morphine on the ability of subjects to estimate intensities of painful stimuli. In their investigation, the pulp nerve of a tooth containing a silver amalgam filling was stimulated electrically at intensities three times the absolute perception threshold value. These stimuli, which served as standards, were definitely painful. After morphine the subjects were required to manipulate rheostat knobs on the stimulating apparatus until the test stimuli were reported as being equal to the standard. (The apparatus was so adjusted that turns of the knob did not correspond with the intensity of stimuli or with dial scale readings.) No change in the accuracy of estimation of painful stimuli was observed following the administration of morphine. However, analysis of the conditions under which the studies were conducted indicates that considerable effort was expended to *reduce* anxiety in the subjects; they were given considerable control of the experimental situation since they manipulated the stimulator knob themselves and applied the electrode to their own tooth fillings. In addition they were trained in estimating intensities of painful stimuli for approximately one week prior to testing. In view of conditions and results discussed in published studies on pain perception thresholds, it might be expected that if conditions had been favorable for *enhancement* of anxiety (anticipatory responses to expected unpleasant stimuli), Isbell and Frank would have obtained different results.

The present investigation is an attempt to determine the extent to which anxiety and morphine alter pain intensity estimation. The experiment was designed to investigate the effect of controlled variations in the experimenter's treatment of the subject on estimation of pain intensities, while the number, the order, and the intensity of the stimuli were kept constant for all groups. If it could be

demonstrated that morphine alters pain intensity estimation under the usual experimental conditions and produces no comparable alteration under circumstances designed to alleviate anxiety, it would be very strongly indicated that morphine exerts a significant influence upon pain intensity estimation only when anticipation of pain is present. The conclusion would then be apparent that failure to consider the variable of anticipation might be responsible for the reported variations in pain perception thresholds following the administration of analgesics.

Specifically, the present report deals with the effects of single analgesic doses of morphine (15 mg. subcutaneously) on the ability of subjects to compare intensities of painful stimuli under two conditions: (*1*) proceeding with the experiment in the usual way without familiarizing the subject with the potentially "fear-inspiring" experimental situation; and (*2*) preceding and accompanying experimentation with measures designed to allay the subject's anxiety. As indicated, it was hypothesized (*a*) that overestimation of intensity would occur under condition (*1*), (*b*) that morphine would significantly reduce this overestimation, (*c*) that little overestimation of the stimuli would occur under condition (*2*), and (*d*) that morphine would exert no significant impairment of estimation under condition (*2*).

METHODS

Subjects. Forty-two white and colored post-addicts (individuals with previous histories of opiate addiction who had not received such drugs for a period of several months prior to the experiment) acted as subjects. They were unselected except that several prospective subjects were disqualified, some because of ill-health, others because of refusal to complete the test, and others because of difficulties with apparatus at the time of testing. Each subject served two days, the sessions being separated by 48 hours. Half of each group received medication on the first day and the other half received it on the second day. Assignment to particular groups was made a week in advance, eliminating contact between experimenter and subject prior to the test. Scheduling in this manner provided two similar groups; in lieu of more appropriate labels one was termed the "Formal" Group and the other the "Informal" Group according to the treatment they would receive.

As indicated, differentiation between the groups was accomplished by the experimenter's attitude toward the subjects and was based upon the degree of formality with which the test was conducted. The so-called "Formal" Group was processed in a manner similar to that employed in the usual experiments on pain. Little explanation of the test procedure or the apparatus was proffered. Actual testing was conducted in a darkened room surrounded by complex electronic equipment. From the time the subject was called to the testing room to the time he was dismissed, the relations between experimenter and subject were strictly impersonal but not unfriendly. In this group electric shocks were administered by the experimenter after a warning light was flashed.

In the treatment of the "Informal" Group a very casual manner was adopted. This consisted of conversing with the subject in the waiting room, describing as much as was feasible of the experiment without divulging its purpose, explaining the operation of the apparatus, having the light on in the testing room during the explanation, demonstrating the method of shocking (the experimenter applying the electrodes and shocking himself), offering the subject cigarettes, and generally attempting to create an atmosphere of congeniality. In this group electric shocks were self-administered by the subject, after a warning light was flashed.

The groups were organized as follows: "Formal" Group with morphine medication (15 mg.), 16 subjects; "Formal" Group with placebo medication (7.5 mg. thiamin hydrochloride[1] dissolved in a volume of distilled water equal to that of the morphine solution used), six subjects; "Informal" Group with morphine medication, 14 subjects; "Informal" Group with placebo medication, six subjects.

Apparatus and procedure. In another study, which has been described in the preceding paper (10), an apparatus was developed to deliver painful electrical stimuli to the fingers of one hand. The general features of this apparatus will be outlined below. In this study it was determined that verbal reports on the intensities of shock stimuli correlated best with wattage indices, rather than those of voltage or amperage. In other words, insofar as the physical aspects were concerned, the effectiveness of the stimulus in evoking a psychological response was determined by its power (wattage) characteristics. Therefore, the effective strengths of all stimuli in these experiments were controlled on the basis of wattage delivered to the subjects.[2]

For present purposes, the general procedure utilized for all subjects may be described as follows. The sub-

[1] In the present studies, a solution of thiamin hydrochloride was used instead of distilled water in order to insure a transient "sting" on subcutaneous injection which resembles that produced by morphine solutions.

[2] The term "power" as used in these experiments refers to calculations made on the basis of the equation $P = I^2R$. As was shown previously (10), such values represent only "apparent" powers since a biological circuit includes not only resistance but capacitance and possibly inductance. However, as has been pointed out (*loc. cit.*), for low values of resistance such "apparent" power corresponds quite closely to actual power delivered.

ject washed his hands before being shown into the quiet, air-conditioned room. Both surfaces of the middle three fingers were cleaned with acetone and thoroughly scrubbed with a tooth-brush coated with electrode paste. (This technique greatly reduced inter-individual differences in skin resistance.) The lateral surfaces of the fingers were wiped with acetone and the electrodes were applied, being fastened in place with a rubber band. By means of an alternating current Wheatstone bridge, a cathode ray oscilloscope, and other instruments, the capacitance in the subject's circuit was balanced out and his skin resistance obtained. Balancing the capacitance before obtaining the resistance was deemed necessary since it is known that the functioning of an organic circuit does not parallel that of a non-organic circuit. Because of this fact, during the development of the apparatus and procedure, empirical charts were constructed that showed the relationship between changes in skin resistance and the voltage that would be required for any specific power. By reference to this chart the experimenter could obtain at a glance the voltage necessary for any desired power at any skin resistance. In this manner a family of wattage curves was employed for pre-setting the stimulator at any desired step of the scale. The scale powers, nine in number, were approximately 0.15 watt, diverging in both directions from a standard of approximately 1.65 watts.[3] This standard power was a definitely painful stimulus when tested by the experimenters upon themselves. Further information on the standards and scale, including errors of prediction, may be found in the preceding report (10).

After a subject's skin resistance had been obtained, six shocks of progressively increasing power were delivered until the standard was reached, and this stimulus was repeated twice. To give the subject practice in comparing stimuli, one complete series of nine shocks was administered in a predetermined random order at approximately 45-second intervals.[4] In accordance with instructions, the subject responded to each shock by reporting verbally whether it was stronger or weaker than the previously given standards. On completing this series the subject was given four more standard stimuli preceded by the following information: he was told to remember the strength of the standards since, after a rest interval of one hour, his judgment would be required on several more series. The subject was then returned to the waiting room and medication was administered, if scheduled.

After one hour the subject was returned to the experimental room, prepared in the same manner as before, and the skin resistance obtained. Six test series, which were the same as the practice series, were then administered; all series were consecutive except that a three-minute rest period was interpolated between the third and fourth. Prior to each of these test series the subject was told to judge each shock with respect to whether it was stronger or weaker than the previously given standards, and he was cautioned not to compare the shocks with each other.

TABLE I

Comparison of group wattage means for the entire test and for separate halves of the test

Group	Control mean	Morphine mean	Mean diff.	Sigma Md	t	P*
Entire "Formal"	1.52	1.65	0.13	0.038	3.42	<1%
Entire "Informal"	1.58	1.63	0.05	0.038	1.32	>20%
1st three series "Formal"	1.53	1.68	0.15	0.042	3.57	<1%
1st three series "Informal"	1.64	1.67	0.03	0.034	0.88	>30%
2nd three series "Formal"	1.52	1.63	0.11	0.061	1.80	>5%
2nd three series "Informal"	1.55	1.65	0.10	0.052	1.92	>5%

* The P column indicates the percentage of such results that would be expected to occur by chance. The 5 per cent level or less is accepted as showing that a significant difference was obtained.

Treatment of data. Two distinct treatments were applied to the data. The first was the simple method of totalling the "stronger" responses for each individual and calculating the significance of the difference between group means by use of the *t* test (Edwards [11]). The second method involved comparisons of mean powers (the mean power levels that the subjects judged as being equal to the standards). Although the error between predicted power and delivered power was usually small, it appeared to be of sufficient magnitude to prohibit a direct transformation from the mean relative percentages of "stronger" judgments given by the Spearman Summation Method to mean powers for each individual, since several equalization processes would have been necessary. A closer correspondence between the subject's report and the statistical statements seemed to be maintained by the following method: (*1*) computing for all subjects the relative percentages of "stronger" judgments by the Spearman Summation Method (Woodworth, [12]);[5] (*2*) transforming the mean relative percentages for each group into power indices and obtaining the "50 per cent points" (the point of subjective equality, *i.e.*, the power that was judged as being equal to the standard); (*3*) calculating the statistical significance of the differences between the means of the various conditions. For purposes of graphic presentation, the mean percentage of "stronger" judgments of each scale step was plotted against the mean power delivered and

[3] For the great majority of subjects nine powers were sufficient to obtain a complete series. However, several individuals required an additional step at either end of the scale.

[4] To lessen the influence of stimulus order on verbal reports, two predetermined random orders were employed, each order being given to half of each group.

[5] Although the Spearman Summation Method does not weight errors, it does afford a rather accurate means of converting "stronger" judgments into power when the scale steps are not entirely equivalent. When scale steps are equal the method advocated by Davis (13) would seem more appropriate.

free-hand smoothing gave the usual psychophysical or ogive curve.

Early in the experiment it became evident that changes in a subject's estimate of the intensities of stimuli occurred frequently as the experiment progressed. Therefore, t tests were made of the differences between the "Formal" and "Informal" Groups, not only for the entire six series but also the first and second halves of the experiment separately.

RESULTS

"Formal" conditions. Table I and Figure 1 present the results obtained on control and morphine days for the "Formal" Group (power calculations). It can be seen that the "50 per cent" point (that point on the curve which the subjects judged to be equal to the standard) for the control, or non-morphine days, is at a considerably lower power than that of the standard. This indicates much overestimation of the intensity of stimuli. The corresponding point on the curve for morphine days is at the standard power of 1.65 watts. This shows that no constant or mean error occurred and that the administration of analgesic doses of morphine under these conditions resulted in the elimination of overestimation. A t value of 3.42 was obtained for the difference between the means of these two conditions; for 15 degrees of freedom this difference would be expected to occur by chance once in somewhat more than 100 repetitions of the experiment. It is quite evident, then, that morphine reduced the tendency to overestimate intensities of painful stimuli under the testing conditions utilized for the "Formal" Group.

Results obtained on the "Formal" Group which had placebo medication will not be presented graphically. The mean for control days was 1.56 watts, and the mean for placebo days was 1.54 watts, giving a non-significant t of 0.29 when compared by the difference method. As might have been predicted, both of these means approximated the mean for the control days of the "Formal" Group which received morphine on medication days. These results show overestimation of the intensities of painful stimuli and no reduction following the administration of the placebo.[6]

[6] The inefficacy of placebos in altering the overestimation of intensities of painful stimuli in the "Formal" Group may be related to the particular type of subjects used in these experiments; post-addicts are sufficiently experienced to recognize the nature of the medi-

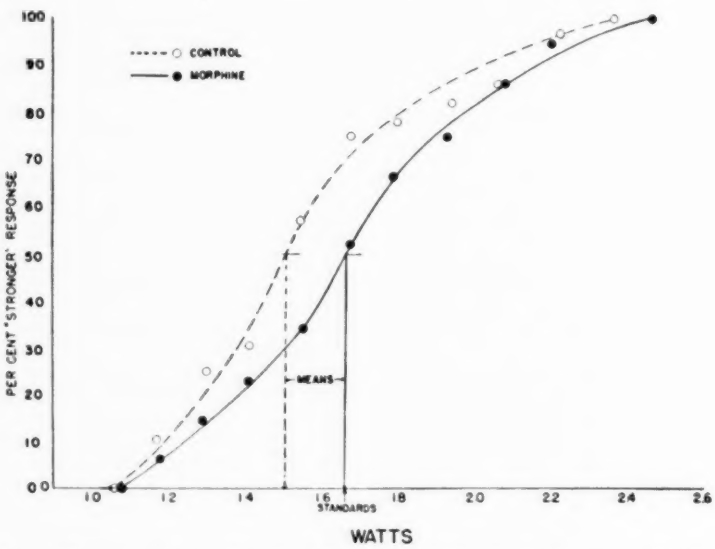

FIG. 1. FREQUENCY OF THE RESPONSE "STRONGER" IN DELAYED COMPARISON OF ELECTRIC SHOCK STIMULI ("FORMAL" GROUP, N = 16)

For explanation, see text.

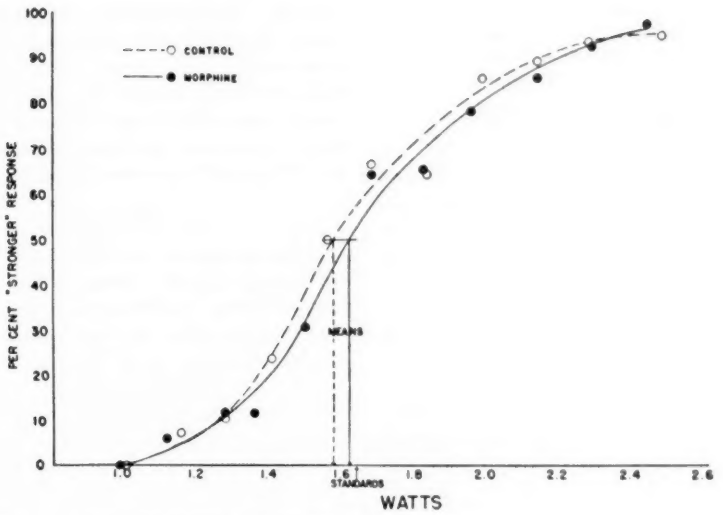

FIG. 2. FREQUENCY OF THE RESPONSE "STRONGER" IN DELAYED COMPARISON OF ELECTRIC SHOCK STIMULI ("INFORMAL" GROUP, N = 14)

For explanation, see text.

"Informal" conditions. Table I and Figure 2 show the results obtained on the "Informal" Group (control, and morphine medication). The "50 per cent point" for control days is somewhat lower than the standard, and the corresponding point for morphine days is also slightly lower than the standard, but approximates it closely. The difference between the means of these two conditions is non-significant. The t of 1.32 for 13 degrees of freedom indicates that this difference would be expected to occur by chance once in four repetitions of the experiment.

As before, the outcome on the placebo group for the informal conditions will not be illustrated. The mean for control days was 1.65 watts, and the mean for placebo days was 1.57 watts, giving a non-significant t of 1.14 for the difference between them. It may be seen that the "50 per cent point" for control days under these informal conditions was at the standard, and that this point for placebo days was at a somewhat lower power level.

Further analysis. In the section on treatment of the data it was mentioned that separate statistical treatments had been given the first and second halves of the six test series. The present hypothesis would be strengthened considerably if a greater difference between control days of the "Formal" and "Informal" Groups were shown to exist for the first three series and if a regression toward more common judgments occurred toward the end of the experiment. This reasoning is based on the assumption that, as the experiment continued, the increasing familiarity of the "Formal" Group with the shock and the general experimental situation would tend to diminish their anxiety. On the other hand, repeated painful shock stimuli might be expected to develop some anxiety in the "Informal" Group.

Table II presents the data on total "stronger" responses for the entire experiment and for the separate halves of the experiment. The first two comparisons, those between sums of "stronger" responses for the entire six series, show that morphine significantly reduced the total number of "stronger" judgments for both groups. After separating the data into first and second halves, it was found that morphine reduced significantly the number of "stronger" judgments for the "For-

cation. It might be expected that in non-sophisticated subjects placebos might have different effects (Wolff and Goodell [14]), since suggestion is known to elevate pain perception thresholds, and to relieve clinical pain.

TABLE II

Comparison of "stronger" responses for the entire test and for separate halves of the test

Group	Control mean	Morphine mean	Mean diff.	Sigma Md	t	P
Entire "Formal"	32.50	26.94	5.56	1.98	2.81	<2%
Entire "Informal"	29.57	26.79	2.79	1.23	2.27	<5%
1st three series "Formal"	16.50	13.13	3.37	1.06	3.18	<1%
1st three series "Informal"	13.78	13.21	0.57	0.67	0.85	>40%
2nd three series "Formal"	16.00	13.81	2.19	1.48	1.48	>10%
2nd three series "Informal"	15.79	13.71	2.08	1.04	2.00	>5%

mal" Group in the first three series ($P < 1$), but had no such effect upon the "Informal" Group ($P > 40$). The difference between the groups is at the 5 per cent level of confidence. These findings show that the experimental procedure was effective and that morphine exerted a significant reduction of overestimation in only the "Formal" Group.

A different outcome occurred in the second halves of the experiment. Although morphine lowered the number of "stronger" responses in both groups the reduction approached significance only in the "Informal" Croup. The difference in total "stronger" responses in the last three series on control days between the "Formal" and "Informal" Groups was not significant ($P > 90$). This shows a trend toward common judgments.

Similar outcomes are shown to have occurred when calculated power means of the separate halves of the experiment are compared, (Table I). (In this connection, it should be remembered that an overestimation of stimuli is inferred when a lower power is estimated as being equal to the standard, *cf.* Figure 1.) As mentioned, the administration of morphine resulted in a significant reduction in the overestimation of the "Formal" Group for the entire six series, but resulted in a slight, non-significant reduction in the estimation of the "Informal" Group. A much greater difference in favor of our hypothesis is observed to occur when the mean powers (mean estimation in terms of watts) of only the first three series are compared. Table I shows that the difference between the control and morphine means of the "Formal" Group was significant at less than the 1 per cent level, and that the difference for the "Informal" Group was non-significant, being greater than the 30 per cent level of chance occurrence. This comparison shows again the very strong influence of the experimental procedure. In the comparisons of mean powers of the second halves of the experiment, the data indicate that the groups approached a more common basis of judgment. This is in agreement with calculation of total "stronger" responses during the same period.

DISCUSSION

Both methods of evaluating the data yielded unequivocal results. Computations based upon total "stronger" responses and those based upon mean powers were in close agreement. These data indicate that when the experiment was conducted under informal conditions, pain intensities were overestimated only slightly and morphine had no significant effect, if any, on discrimination. In contrast, marked overestimations of pain intensities occurred under formal control conditions and morphine practically abolished this error. In effect, therefore, morphine enabled the "anxious" groups to estimate the intensities of painful stimuli as well as the group in which anxiety had been dissipated. That this effect was due to a specific action of morphine is indicated by the fact that administration of placebos had no significant effect upon the estimation of painful stimuli in either group.

From these results it may be inferred that ordinary formal experimental conditions tend to produce anxiety which promotes overestimation of the intensities of painful stimuli. The effects of morphine, as shown in these experiments, therefore, can be interpreted as due to a reduction of anxiety.

Furthermore, it has been shown that morphine tends to relieve anxiety which is produced by two different types of experimental situations. Considering the results for the first half of the test for the "Formal" Group, it was shown that anticipatory reactions to the unknown aspects of the experimental situation resulted in significant overestimation of pain intensities and that morphine significantly reduced this overestimation. It may be inferred that in this group anxiety lacked a clear and definite object. However, it was shown also that overestimation was induced in the "Informal" Group during the second half of the test, and that administration of morphine resulted in a reduction of this error, which was close

to being significant. The overestimation of this group seemed to be due to previously applied shock. Both of these types of anxiety, namely, with and without a definite object, were relieved by morphine.

However, there is evidence that not all types of anxiety are reduced by morphine. For example, in subjects who exhibited suspicion and hostility toward the experimenters, morphine had little effect on the overestimation of intensities of painful stimuli. Therefore, it would appear that although morphine reduces anticipatory fear of pain, it may not act as a general anxiety relieving agent. This inference is supported by data obtained from a psychodynamic study of an addicted patient by Wikler (15) which indicate that morphine reduces such anxiety as is related to inadequate gratification of so-called "primary" needs, such as hunger, fear of pain and sexual (general erotic) urges, while the drug has little effect on such anxiety as is related to inadequate gratification of "secondary" needs, such as narcissism, dependence, etc.

That "emotional factors" may influence pain threshold measurements and the effects of drugs thereon has been suggested and demonstrated by several investigators; this aspect has been discussed in the review by Wikler (1). Judging from our present studies, it appears that anticipatory fear of pain is one such important variable which must be controlled in investigations of this sort. Failure to do so may account, in part, for the wide discrepancies which have been reported by different observers in such investigations.

Because of its considerable theoretical importance in relation to the problem of pain, a more direct method for quantitative measurement of the effects of anticipatory fear of pain has been devised. This will be described in detail in another communication (16).

SUMMARY AND CONCLUSIONS

1. The effects of subcutaneous injections of 15 mg. of morphine on the ability of subjects (post-addicts) to judge the intensity of painful electric shock stimuli were studied under two conditions: (*a*) under formal conditions, proceeding with the experiment without familiarizing the subjects with the potentially fear-inspiring experimental situation; (*b*) under informal conditions, preceding the experiment with reassurance, demonstration and explanation designed to allay the subject's anxiety. In both groups six consecutive series of nine stimuli were delivered in each experiment, and the subjects were required to state whether each stimulus was "stronger" or weaker than a standard stimulus. The strength of each stimulus was controlled on the basis of the power (wattage) value delivered to the subject.

2. During the first three series under formal control conditions, a significantly greater number of test stimuli were judged "stronger" in comparison with the standard stimuli than were actually delivered, and the power (wattage) values of the test stimuli at the points of subjective equality (50 per cent point) were lower than those of the standard stimuli. These errors were reduced significantly by administration of morphine, but were not altered by placebos.

Under informal control conditions, verbally reported judgments of test stimuli were extremely accurate and power values of the test stimuli at the points of subjective equality were practically identical with those of the standards. Neither morphine nor placebos had any significant effect on the estimation of the intensities of painful stimuli.

3. Under control conditions during the last three series the accuracy of estimation of the intensities of painful stimuli remained practically unchanged in the "Formal" Group but deteriorated in the "Informal" Group. In both groups morphine tended to reduce the magnitude of error, while placebos had no apparent effect.

4. It is concluded that (*a*) under conditions which promote anxiety or fear of pain, subjects tend to overestimate the intensities of painful stimuli; (*b*) morphine reduces such anxiety; (*c*) under conditions in which anxiety is largely eliminated, little if any overestimation of the intensities of painful stimuli occurs; (*d*) morphine does not affect the ability of subjects to accurately estimate the intensities of painful stimuli when anxiety is dissipated; and (*e*) anxiety, particularly that which is associated with anticipation of pain, is one important variable which must be controlled in experimental investigations of problems related to pain and analgesia.

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HORMONE EXCRETION IN LIVER DISEASE[1]

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Gynecomastia, cutaneous spider nevi, testicular atrophy, and loss of libido are frequently found in men with chronic liver disease. The clinical aspects of the subject have recently been reviewed (1). On the basis of studies of urinary estrogen excretion (2), these changes have been attributed to increased levels of estrogens, particularly unconjugated estrogens presumably resulting from decreased conjugation in the diseased liver. This presumption is based on abundant evidence indicating that the liver of certain laboratory animals may "inactivate" estrogens (3), and that the subcutaneously injected conjugated estrogens are less "potent" than the free forms (4). However, more recent evidence indicates the role of the human liver in estrogen inactivation is less impressive (5) and that the relative inactivity of conjugated estrogens is not apparent when tested at the target tissue level (6, 7).

Figure 1 shows in diagrammatic fashion some of the factors usually considered in the production and metabolism of estrogens in man. Several authors (3, 8–10) have recently reviewed the metabolism of estrogens, therefore the large amount of data supporting this scheme need not be cited here. The importance of the liver in the metabolism of estrogens is apparent. It will be noted that, if production is constant, the estrogen level of the blood may presumably be modified through: (*a*) excretion of the material in the urine and bile (and through the intestinal wall?) some being lost in the feces despite an enterohepatic circulation, (*b*) destruction of the estrogen molecule, and (*c*) production of conjugated estrogens and of estriol, forms that are usually considered "less potent."

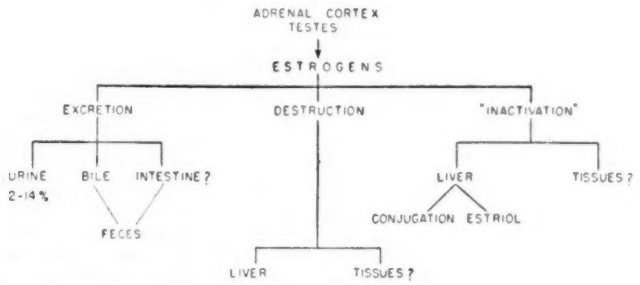

FIG. 1. ESTROGEN METABOLISM

This figure outlines some of the factors that have been given consideration in discussions of the metabolism of estrogens.

With these concepts in mind, we have analyzed the urinary excretion of the unconjugated and conjugated forms of the "estradiol," "estrone," and "estriol" fractions as well as gonadotrophins and neutral 17-ketosteroids for one or more periods in five normal men and 17 men with diffuse hepatic disease. The results are presented in

[1] This investigation was conducted under the auspices of the Commission on Liver Disease, Armed Forces Epidemiological Board, and supported in part by the Office of The Surgeon General, Department of the Army, Washington, D. C.

relation to: (*a*) the severity of the liver disease, (*b*) the standard liver function tests, (*c*) the effect of liver disease on estrogen metabolism, (*d*) the inter-relationships of urinary hormone excretion, and (*e*) the presence or absence of gynecomastia, cutaneous spider nevi, palmar erythema, testicular atrophy, and loss of libido. In addition, data concerning the urinary excretion of unconjugated neutral 17-ketosteroids, the ketonic and non-ketonic steroid fractions, and the urinary corticoids are briefly presented.

MATERIALS AND METHODS

Clinical material. Of the special group of 17 patients with diffuse liver disease whose urine was subjected to estrogen fractionation studies, 15 were studied in the Hepatic and Metabolic Section at the Valley Forge Army Hospital and two were studied on the Medical Wards of the Hospital of the University of Pennsylvania. Results reported under miscellaneous observations were, in part, obtained on patients other than those in this special group. These patients were also studied at these two hospitals. Special aspects of methods concerning them will be mentioned in the text. In most instances, patients were selected for this study because of the severity of the symptoms or the presence of gynecomastia, testicular atrophy or spider nevi. The five controls were healthy men without known liver disease.

Chronic hepatitis was diagnosed in four of the subjects, subsiding acute hepatitis in one, "portal cirrhosis" in 11, and one had severe hemochromatosis. The term chronic hepatitis, as used in this paper, indicates that the patient had a history compatible with that of acute viral hepatitis, with "recurrence" or incomplete recovery six months or more after the acute episode. The term "portal cirrhosis" is used to indicate a diffuse hepatic fibrosis in men usually associated with a history of alcoholism, but without a history suggestive of acute viral hepatitis.

Frequent clinical observations and tests of liver function and a needle biopsy of the liver were obtained. The severity of the liver disease was then classified as 0 to 4 plus in each case. This classification was made by a numerical evaluation of: (*a*) severity of symptoms and physical findings, (*b*) results of liver function tests, and (*c*) histologic appearance of liver biopsies.

The liver function tests were done by standard technics and those used for the above classification included total serum bilirubin, thymol turbidity, 24 hour cephalin cholesterol flocculation, prothrombin activity, percentage of cholesterol in the esterified form, serum albumin and the measurement of bromsulphalein retention if the serum bilirubin was less than 1.3 mg./100 ml. These values were scored 0 to 4 plus and averaged as were the histologic and clinical criteria of liver disease. The final estimate of the severity of the disease was then based on an average of the estimate in each of the three categories.

The grade of gynecomastia was estimated as 1 to 4 plus on the basis of unilateral or bilateral involvement, the size of the firm "button" of tissue beneath the areola, and the presence or absence of tenderness.

Cutaneous spider nevi have been classified as 1 to 4 plus according to number, size, presence or absence of pulsations, and location. Nevi located about the neck and face were not considered as significant as those located about the trunk and extremities, since small spider nevi are frequently found in the former sites in "normal" individuals.

Scrotal contents of all patients were palpated to determine testicular size and consistency. Measurements of length and width were obtained with the surrounding scrotal wall thinned maximally without distortion of testicular dimensions. Testicular atrophy was considered present when overall length measured less than 4.5 cm. and overall width less than 3 cm. The three patients classified as having marked atrophy had soft testes considerably smaller than the lower limits of normal.

Hormone assays. Urine collections were made under the supervision of trained personnel. In a few instances, the periods of collection were three days; all others were six to 22 days. This quantity of material permits considerably more accurate bioassays than a single 24 hour collection and, in addition, provides a more representative sample. All urine was preserved with toluene and prompt refrigeration. The few daily urine collections with pH of 7 or greater were acidified to pH 6 to 6.5 shortly after the 24 hour collection was completed. Extractions were completed within 48 hours of the last day of the period. Aliquots were taken from the daily urine specimens so that a representative sample was obtained for gonadotrophins and 17-ketosteroids. Daily creatinine determinations were made as a check on the accuracy of the urine collections in 17 of the total of 26 periods. The term "hormone" in this presentation, following conventional usage, includes hormone metabolites.

Estrogens. The unconjugated (free) estrogens were extracted for 48 hours at pH 6–7 using the continuous ether extraction method of Wilson, Grauer, and Saier (11). After hydrolysis for one hour at 95° C. with 5%, by volume, of concentrated sulfuric acid, the conjugated form of the estrogens was similarly extracted. These two extracts were then separated into the "estrone," "estradiol," and "estriol" fractions by the method of Friedgood, Garst, and Haagen-Smit (12). This procedure was modified to include the Mather (13) technic of adjusting the pH of aqueous solutions of estrogen to pH 9 before extractions with ether. Thus, six fractions were obtained; representing the free and the conjugated forms of the "estrone," "estradiol," and "estriol" fractions.

Bio-assays of the estrogen fractions were performed in estrone primed, pure strain, castrate female white mice (CFW 1). Estrone and estradiol fractions were given each morning, and the estriol fraction twice daily, subcutaneously in corn oil for three days. Vaginal smears were made at 72 and 96 hours after the first injection and were stained with methylene blue. After preliminary as-

say of each unknown employing six or more mice, a "final" 2-point assay was made using from four to 40 mice (mean = 13). The number used depended upon the amount of "estrogen" available in the "unknown" as indicated by the preliminary assay. The bio-assay of each group of unknowns of the same fraction was always accompanied by a two-dose level control assay (40 mice) of the appropriate standard estrogen, *i.e.*, crystalline estrone, estradiol, or estriol. The quality control test of Simon (14) allowed us to infer that the results of these control assays with any single crystalline estrogen were homogeneous because the subsample values fell satisfactorily within the 0.01 control lines. In addition, the responses for estrone were fitted to a binomial curve and again the discrepancies between the theoretical and observed distributions were attributable to chance variation ($P = 0.18$ for the low dose, $P = 0.75$ for the high dose). Since there was no statistically valid demonstration of significant variation in the sensitivity of the mice, the results of all controls were pooled. This lack of variation in sensitivity of the mice is not in agreement with the experience of Emmens (15) and may be due to differences in technical details. The results of the assays of the unknowns have been compared with the pooled results of the standard. The relatively small estimated error of the standard is reflected in the estimated error (limits of confidence) of the unknowns. The potency estimates and the limits of confidence in 39% of the 156 assays were calculated by the probit analysis method of Emmens (16). In these instances, sufficient estrogen was usually present in the urine so that a two-dose level assay, using 10 to 20 mice per point, could be performed. The mean of the dose response slope (b) of the urine extracts was approximately 5.7. That of the crystalline standards was 6.7. Parallelism of the dose response lines of the unknown to the standard was assumed in those few instances where statistical evidence indicated more than one chance in 20 that this was not so. The limits of confidence of all assays have been calculated at the 95% confidence level. For those assays in which probit analysis was not employed, the limits of confidence were calculated from Mainland's Table of Confidence Limits for Enumeration Data (17).

"Estrone Equivalent" is the term used to indicate estrogenic effect expressed as the amount of crystalline estrone required to produce a similar effect under similar conditions. We have calculated it as the sum of the median values (or the potency estimates of the probit analysis data) for each of the estrogen fractions expressed in terms of the amount of estrone with equivalent estrogenic activity. This is obtained from the relationship of the ED_{50} of estradiol (0.043 μg.) and estriol (0.289 μg.) to estrone (0.142 μg.). The ED_{50} is that amount of each of the crystalline estrogens necessary to produce full cornification of the vaginal smear in 50% of the mice used in the control assays. The term total estrogens indicates the sum of the estrone equivalents of the free and conjugated forms of all three fractions.

Critique of method for determination of estrogens. Since the separation and determination of the estrogen fractions is a difficult task about which there is considerable difference of opinion, we have extracted and analyzed one 22 day collection of urine (patient No. 1) in duplicate. Each daily specimen was divided into halves (A and B) and aliquots taken from each in the usual fashion for gonadotrophins and 17-ketosteroids. The estrogen content calculated from the median values of the confidence limits for each fraction (or the potency estimates in the case of probit analysis) is given below.

Specimen	μg. per 24 hours					
	Estrone		Estradiol		Estriol	
	Conj.	Free	Conj.	Free	Conj.	Free
A	0.51	0.36	0.18	0.06	1.80	0.40
B	Lost	0.29	0.17	0.08	1.76	0.50
Average		0.325	0.175	0.07	1.78	0.45
Deviation from aver.		0.035	0.005	0.01	0.02	0.05
% deviation from aver.		10.8%	2.9%	14.3%	1.1%	11.1%

In addition, 4,500 ml. of urine, representing that excreted during 115 hours by normal control IV, were extracted twice and 30 μg. of estrone were added. One-half of this twice-extracted urine was subjected to hydrolysis and the entire fractionation procedure; the other half was simply rehydrolyzed and extracted with ether and a bio-assay performed. By this latter procedure, estrogenic activity equivalent to 72% of that of the added estrone was found. The bio-assay of the "estrone," "estradiol," and "estriol" fractions showed estrogenic activity of approximately 41%, 13%, and 10%, respectively, of that of the added estrone. This indicates incomplete separation of the small amount of estrone added to the urine. Further evidence that our extraction procedure extracts approximately 60–70% of the small amount of extractable estrogen present in male urine is afforded by the fact that the second extraction of the original urine (before addition of estrone) yielded additional estrogenic material. That extracted on the first extraction represented 66% of the total of the two.

It is concluded from these studies that reasonable agreement was obtained in the duplicate analyses and that recovery and fractionation is adequate to demonstrate major quantitative and qualitative variations. The relatively small calculated error of the duplicates is due, in part, to the use of the pooled values for the controls with its considerably smaller error than that of a single control assay.

Urinary gonadotrophin excretion. Urinary gonadotrophins were precipitated by alcohol and extracted by a modification of the dialysis method of Klinefelter, Albright, and Griswold (18). The bio-assays were performed by measuring the increase in uterine weight of 8 to 10 gm. pure strain immature female mice. After preliminary assays, four mice per dose level were used for final assay. One mouse unit of "gonadotrophin" is considered to be present if the mean uterine weight of the injected mice is increased 100% above the mean uterine

TABLE I

Summary: hormone excretion in men with liver disease

CASE NO.	AGE	DIAGNOSIS	DURATION OF SYMPTOMS	GYNECOMASTIA	SPIDER NEVI	PALMAR ERYTHEMA	TESTIC. ATROPHY	LOSS OF LIBIDO	ESTROGENS* FREE	ESTROGENS* FREE & CONJ.	17-KETO STEROIDS	GONADOTROPHIC HORMONES
	YRS.		MO.						μ G/24 HRS.		MG/24 HR.	M.U./24 HR.
						CONTROLS						
I	25	NORMAL	—	—	—	—	—	—	1.4	4.0	16.2	26
II	27	NORMAL	—	—	—	—	—	—	0.5	2.9	11.6	16
III	27	NORMAL	—	—	—	—	—	—	0.8	3.2	10.0	17
IV	28	NORMAL	—	—	—	—	—	—	0.3	2.9	13.1	28
V	37	NORMAL	—	—	—	—	—	—	0.2	1.7	9.1	25
Aver. 28.8 (25 yr.–37 yr.)									0.6	2.9	12.0	22
S. E. of MEAN									± 0.2	± 0.4	± 1.3	± 2
						GRADE I LIVER DISEASE						
3	24	AC. HEPAT.	5	↑↑↑	0	0	0	0	0.8	12.3	4.0	—
4	26	CHR. HEPAT.	48	0	↑↑↓	↓	0	0	1.0	7.3	19.9	—
5	27	CHR. HEPAT.	11	0	↑↑	↑↑	0	0	0.5	3.9	13.8	21
12	43	PORT. CIRR.	11	↑↑↓	0	0	0	0	0.4	2.5	13.1	15
Aver. 30 (24 yr.–43 yr.)									0.7	6.5	12.7	18
S. E. of MEAN									± 0.1	± 2.2	± 3.3	—
						GRADE II LIVER DISEASE						
1	19	PORT. CIRR.	?	—	↑↑	0	—	—	0.7	2.7	7.3	29
2b	22	CHR. HEPAT.	14	0	↓	0	0	0	1.0	4.6	12.7	27
9	36	PORT. CIRR.	36	0	↓↓	0	0	0	0.3	2.4	8.0	45
10c	37	PORT. CIRR.	52	↑↓	↓↓	0	↑↑	↑↑↑	0.7	5.4	7.7	16
16	53	PORT. CIRR.	288	↑↑↓	↓	0	↓	↑↑↑↑	0.7	6.3	5.3	5
Aver. 33.4 (19 yr.–53 yr.)									0.7	4.3	8.2	24
S. E. of MEAN									± 0.1	± 0.8	± 1.2	± 7
						GRADE III LIVER DISEASE						
6	32	PORT. CIRR.	16	0	0	0	0	0	0.4	2.9	8.7	35
8b	35	PORT. CIRR.	4	↑↑↑	0	0	**	↑↑↑↑	0.3	5.3	8.8	—
10b	37	PORT. CIRR.	49	↑↑↓	↑↑↑↓	0	↑↑↑	↑↑↑↑	3.0	4.7	5.3	3
11	43	CHR. HEPAT.	96	0	↑↑	↑↑↑	0	↓	1.9	5.8	7.8	—
13	46	PORT. CIRR.	48	0	↑↑	0	0	?	0.5	4.1	7.0	39
14	48	HEMOCHROM.	276	0	↑↑	?	↑↑↑↑	↑↑↑↑	1.9	2.7	8.0	3
Aver. 40.2 (32 yr.–48 yr.)									1.3	4.3	7.6	20
S. E. of MEAN									± 0.5	± 0.5	± 0.5	± 10
						GRADE IV LIVER DISEASE						
2a	22	CHR. HEPAT.	14	↑↑↑↑	↓	0	0	0	0.8	7.6	7.9	20
7	34	PORT. CIRR.	5	0	↑↑↑↓	0	0	?	4.3	10.6	8.9	21
8a	35	PORT. CIRR.	3	↓↑	0	0	0	↑↑↑↑	0.6	8.2	5.5	3
10a	37	PORT. CIRR.	48	↑↑↑↓	↑↑↑↑↓	0	↑↑↑	↑↑↑↑	0.3	2.6	5.1	18
15	53	PORT. CIRR.	288	↓	↓	0	**	↑↑↑↑	2.8	12.6	4.6	5
17	60	PORT. CIRR.	3	↓↓	↑↑	0	↑↑↑	?	2.3	6.4	6.0	17
Aver. 40.2 (22 yr.–60 Yr.)									1.9	8.0	6.3	14
S. E. of MEAN									± 0.6	± 1.4	± 0.7	± 3

In general, these patients were selected for study because of the presence of gynecomastia, testicular atrophy, or spider nevi. Eleven patients were considered to have portal cirrhosis; four, chronic hepatitis; one, subsiding acute hepatitis; and one, hemochromatosis. Patients have been grouped according to the severity of the liver disease (see Methods). Three were studied during more than one period. During the interval between urine collections, the severity of the liver disease changed. The sequence of the studies is indicated by the letters. Arrows pointing upward indicate increased severity, and those pointing downward indicate decreased severity.

* Estrogens are expressed in terms of estrone equivalent.

** Scrotal edema.

weight of the control mice. With but few exceptions, no assays are reported in which the increase in uterine weight was less than 37% or greater than 200% above the control. Results within these narrow limits were then calculated from a standard dose response curve which had been calculated from a 5-point assay using 12 mice per point injected with an extract of menopausal urine.

Critique of gonadotrophin method. An estimate of the reproducibility of this method was obtained from 20 duplicate assays. Fifteen were performed at the same time and in five instances, the precipitate was saved and

re-assayed six months later. The average deviation from the mean of the duplicates was 7% and the greatest deviation was 14%. In addition, a duplicate precipitation, extraction and assay were done on the collection from patient No. 1. The duplicates in this case varied 5% from their mean. It is concluded that the assay method as outlined is fairly accurate and consistent.

The total neutral 17-ketosteroids were determined by a modification of the Holtorff-Koch method (19) on an aliquot of the total collection or on an aliquot of each of three or more 24 hour urine specimens, and the results were averaged. Extractions were done with carbon-tetrachloride. *Corticoids* were extracted and determined as neutral lipid-soluble reducing substances by a modification of the method of Heard, Sobel, and Venning (20). Total ether extractable *ketonic and non-ketonic steroids* were determined by weighing these fractions after the Girard separation. The free (pre-hydrolysis) 17-ketosteroids were determined on the neutral fraction of the ether extract.

Statistical methods. Calculation of estrogen assays is indicated above. The analysis of the statistical significance of the difference of means was done by the pooled variance method described by Snedecor (21). Calculations of the regression coefficients and their significance also followed the methods of this author. The term *significant* indicates that there is less than one chance in 20 ($P < 0.05$) that the regression or difference of means is due to chance variation. *Highly significant* indicates less than one chance in 100.

RESULTS

Hormone excretion and severity of liver disease

Table I lists certain clinical data and the values for the urinary excretion of the free (unconjugated) and total (free plus conjugated) estrogens, 17-ketosteroids, and gonadotrophins. Patients have been grouped into four grades according to the severity of the liver disease as judged by the numerical system detailed in Methods.

In 17 of the 21 periods (14 men) the 17-ketosteroid excretion was lower than the lowest normal value, while in six periods (six men) the free form of estrogen, and in 14 periods (11 men)

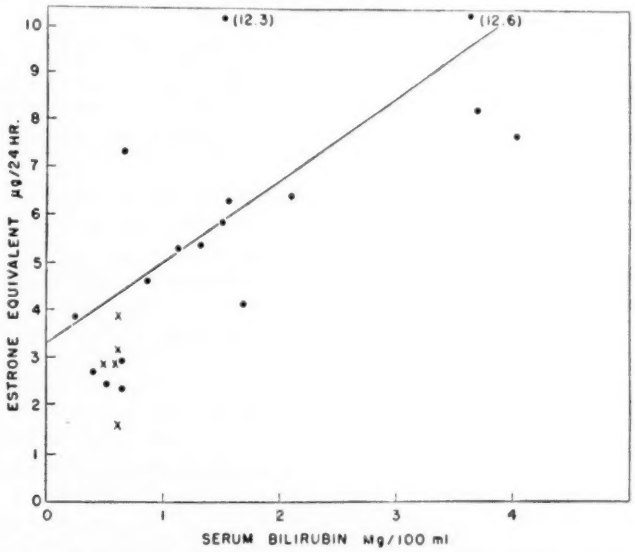

FIG. 2. THE RELATIONSHIP OF THE URINARY EXCRETION OF TOTAL ESTROGENS TO TOTAL SERUM BILIRUBIN

The regression coefficient ($b = 1.71$ with a standard error of 0.50) and the correlation coefficient ($r = 0.65$ with a standard error of 0.19) have been calculated from the values for patients with liver disease, indicated by ●. Values for estrogen excretion in men without liver disease are indicated by symbol ×. Average normal serum bilirubin values are assumed for the control group. This relationship of estrogen excretion and total serum bilirubin is apparently limited to patients without severe jaundice (see text).

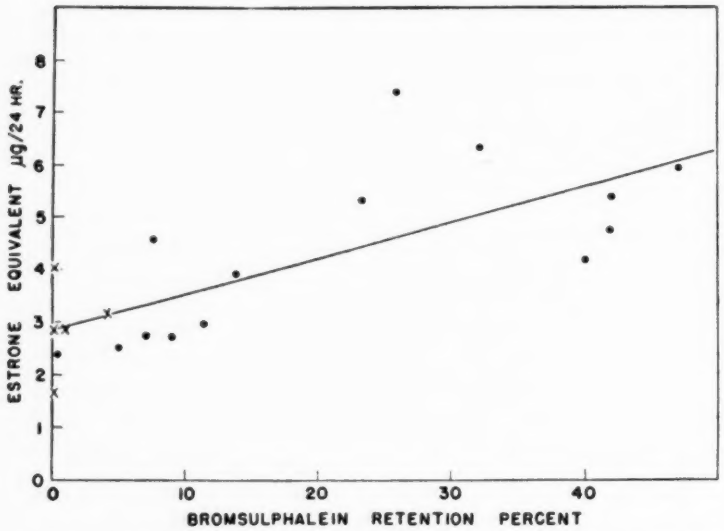

FIG. 3. THE RELATIONSHIP OF THE URINARY EXCRETION OF TOTAL ESTROGENS TO BROMSULPHALEIN RETENTION

The regression coefficient ($b = 0.07$ with a standard error of 0.02) and the correlation coefficient ($r = 0.70$ with a standard error of 0.21) have been calculated from the values for patients with liver disease, indicated by ●. Values for total estrogen excretion in men without liver disease, indicated by x, are plotted along the vertical axis for comparison. In one of the normal men, a bromsulphalein retention test was performed because of a history of hepatitis four years before. This value was 4%.

the total estrogen excretion was higher than the highest normal value. The patients with the most severe liver disease (grade IV) show the greatest excretion of free and total estrogens and the lowest values for 17-ketosteroid and gonadotrophins. Examination of the data shows that the increase in age of this group does not, in itself, account for the decreased 17-ketosteroids nor the elevated estrogens.

Estrogen excretion and liver function tests

All liver function tests were performed during the period of urine collection. Figures 2 and 3 show the highly significant correlation of urinary total estrogen excretion with the total serum bilirubin and bromsulphalein retention. This relationship is apparently valid only for lesser degrees of jaundice. Two observations were made in one patient (No. 10) one and five weeks after recovering from hepatic coma. During these periods, he had total serum bilirubin values of 34 and 11 mg./100 ml., and his total estrogen excretion was 2.6 and 4.7 µg./24 hr. Three months later his serum bilirubin was 1.3 mg./100 ml., and the total estrogen excretion was 5.4 µg./24 hr. It is obvious that the regression demonstrated with mild elevations of serum bilirubin was not evident in this patient with severe jaundice. Furthermore, in a man with obstructive jaundice from carcinoma of the head of the pancreas, who is not included in this series, the total estrogen values were within normal limits when the total serum bilirubin was 20 mg./100 ml. Both men were critically ill. The excretion of the free form of estrogens in the urine did not show a significant correlation with the serum bilirubin or bromsulphalein retention.

The relationship of total estrogen excretion to the cephalin-cholesterol flocculation, Kunkel gamma globulin, serum globulin, serum albumin, and thymol turbidity tests was also examined. No statistically significant relationship was found.

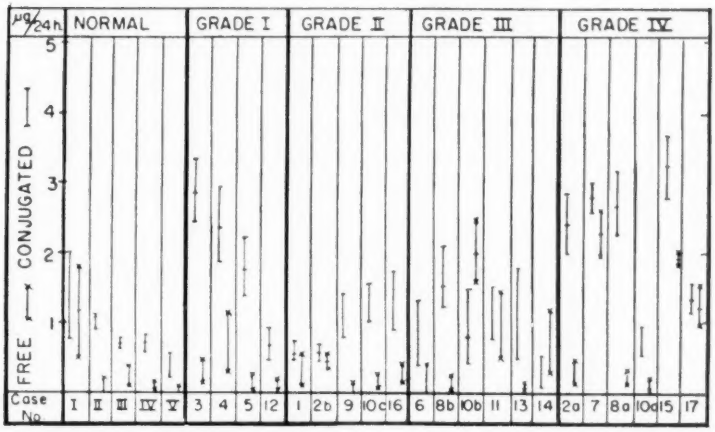

FIG. 4a. URINARY EXCRETION OF FREE AND CONJUGATED "ESTRONE" IN NORMAL MEN AND MEN WITH LIVER DISEASE

Patients have been classified on the basis of a numerical evaluation of clinical, laboratory, and liver biopsy data. Grade IV indicates the most severe degree of liver disease. Results are arranged within each grade in order of increasing age of the patient. The sequence of repeated tests in patients No. 2, 8, and 10 is indicated by the letters a, b, and c. The extremes of the lines |———| and x———x represent the 95% confidence limits of the bioassays. The additional crossbar |——|——| and x——|——x represents the estimate of the potency by probit analysis (see Methods).

These findings, in association with the results presented in Table I, lead to the conclusion that within a limited range the increased estrogen excretion is more closely associated with evidence of impaired "excretory function" of the liver than with the other tests employed or the general severity of the disease as judged by the multiple criteria used in this study.

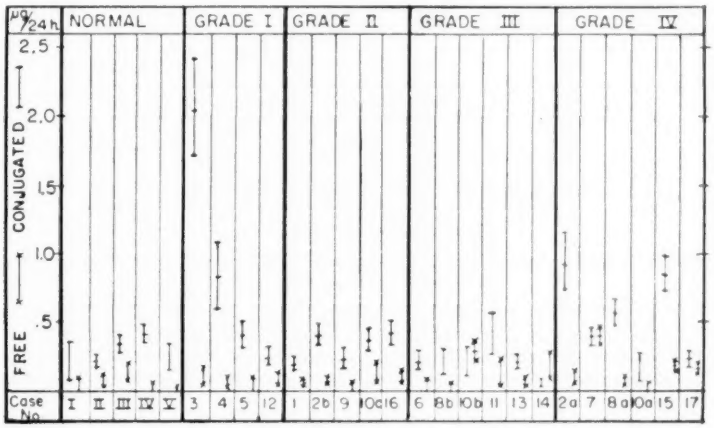

FIG. 4b. URINARY EXCRETION OF FREE AND CONJUGATED "ESTRADIOL" IN NORMAL MEN AND MEN WITH LIVER DISEASE

See Figure 4a for legend.

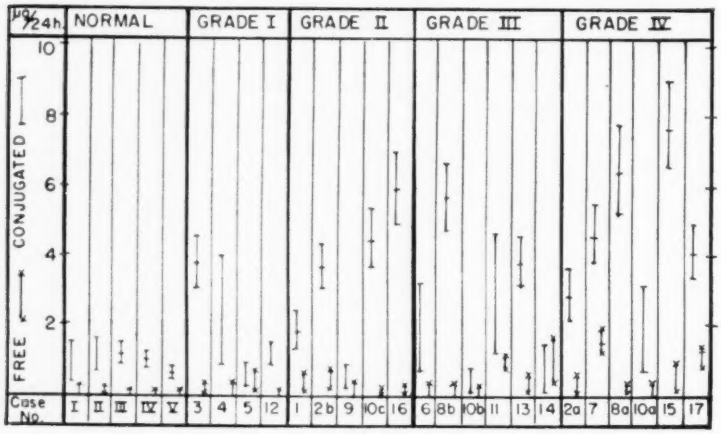

FIG. 4c. URINARY EXCRETION OF FREE AND CONJUGATED "ESTRIOL" IN NORMAL MEN AND MEN WITH LIVER DISEASE

See Figure 4a for legend.

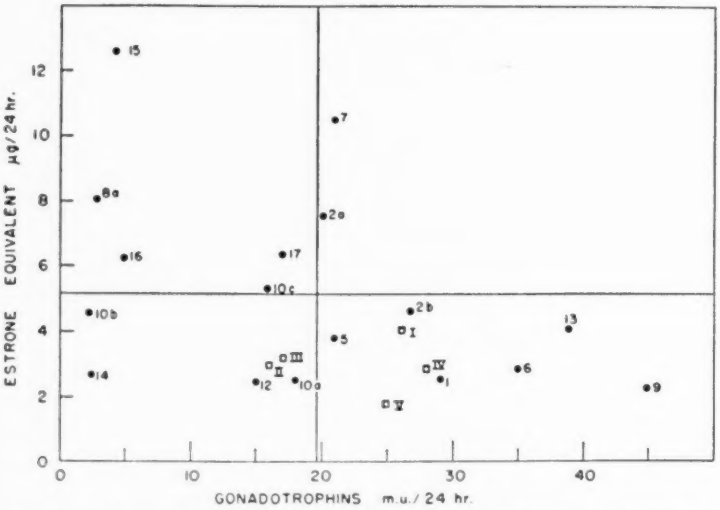

FIG. 5. THE RELATIONSHIP OF URINARY ESTROGEN EXCRETION TO THE URINARY EXCRETION OF GONADOTROPHINS

The numbers indicate the patient and period number (see Table I). Normal controls are indicated by Roman numerals. The crossed lines indicate the mean estrogen and gonadotrophin values for the group. It is evident that gonadotrophin values more than slightly above the average are associated with estrogen values less than average for the group.

The error of the slope of the calculated regression line (not shown) is such that P is slightly greater than 0.05. Patient No. 14 had hemochromatosis. If this value is omitted because of clinical variation from the rest of the series, a "significant" regression is obtained.

Estrogen metabolism and liver disease

Figure 4 a, b, and c shows the excretion of the various estrogen fractions in normal men and men with liver disease. The conjugated form of one of the three fractions showed no overlap of confidence limits with the highest normal value in 20 instances. The conjugated form of "estrone" was increased as judged by this standard in five periods, "estriol" in 11, and "estradiol" in only four periods. The unconjugated form of "estrone," "estriol," and "estradiol" was increased in two, four, and two periods, respectively, a total of eight instances. In three instances, the unconjugated form of a particular fraction was increased without significant increase in the conjugated form. In five instances, there was a considerably greater increase in the conjugated form of the same fraction. Increase in the free form of one fraction was not necessarily associated with an increase in the free form of the other fractions. It is concluded from the above that increase in excretion of the conjugated form is, in general, more frequent and greater than that of the unconjugated form. Increases occur in all three fractions, but in the greatest number of patients and most markedly in both the free and conjugated forms of the estriol fraction.

Inter-relationships of urinary hormone excretion

Figures 5, 6, and 7 show the inter-relationships of urinary hormone excretion values. Figure 5 shows that in those periods exhibiting urinary gonadotrophin excretion greater than the group average, there were only two with estrogen excretion greater than average and nine with less than average estrogen excretion. Thus, high estrogen excretion was associated with gonadotrophin values less than, or only slightly above the average. The free estrogen excretion showed a similar relationship to gonadotrophin excretion (not charted).

Figure 6 shows that only one of the eight ketosteroid values above the average for the group was associated with estrogen excretion values greater than average. In other words, if the 17-ketosteroid excretion was above average, the estrogen excretion tended to be below average.

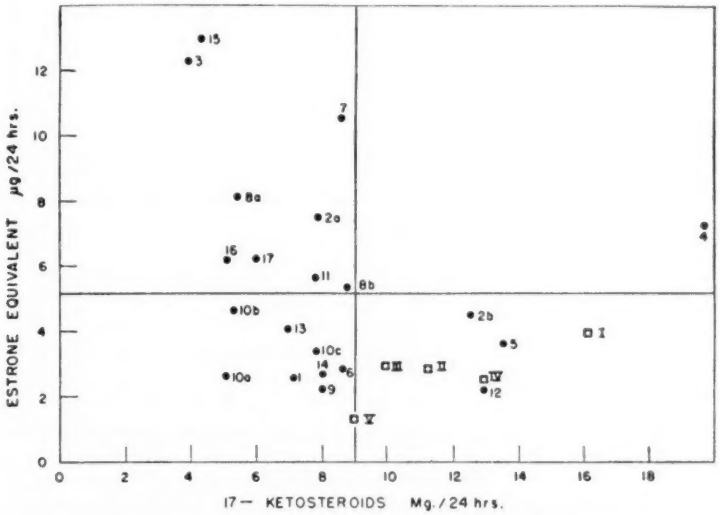

FIG. 6. THE RELATIONSHIP OF URINARY ESTROGENS AND 17-KETOSTEROIDS

The numbers indicate the patient and period number (see Table I). Normal controls are indicated by Roman numerals. The crossed lines indicate the mean estrogen and 17-ketosteroid values for the group. Rank correlation analysis indicated a correlation that was not significant at the $P = 0.05$ level but was at the $P = 0.1$ level.

Figure 7 shows that as the 17-ketosteroid values tended to be approximately average or above, the gonadotrophin values were also approximately average or above. The relationship of these two excretion values to each other is not as definite as their relationship to estrogen excretion.

The relationship of urinary hormone excretion to gynecomastia, cutaneous spider nevi, palmar erythema, testicular atrophy, and loss of libido

Gynecomastia. Figure 8 shows that the total estrogen excretion in the four men with stable or advancing gynecomastia was significantly greater than that for men with liver disease but without gynecomastia, the four men with regressing gynecomastia, and the normal controls. The four men with gynecomastia which disappeared within four months had a mean total estrogen excretion approximately the same as patients without gynecomastia. The increase in total estrogens in the men with stable or advancing gynecomastia was due to significant increases in the conjugated form of all three fractions. The unconjugated form was not significantly increased above that of patients with regressing gynecomastia, or none at all.

The mean and S.E. of the mean for the neutral 17-ketosteroid excretion was 5.9 ± 1.0 mg./24 hr. in patients with stable or advancing gynecomastia; 7.6 ± 1.8 mg./24 hr. in patients with regressing gynecomastia; and 10.2 ± 1.3 mg./24 hr. in patients without gynecomastia. This relationship is reflected in the estrogen/17-ketosteroid ratio also shown in Figure 8. The average gonadotrophic hormone excretion (not charted) was significantly reduced in the eight patients with gynecomastia to 11 m.u./24 hr. compared to 22 m.u./24 hr. for the controls and 28 m.u./24 hr. for the patients without gynecomastia.

Cutaneous spider nevi. No significant relationship was found between the urinary excretion of total estrogens, free estrogens, 17-ketosteroids, or gonadotrophins and the presence of cutaneous spider nevi. The only significant increase is that of the free form of the estriol fraction in the 10

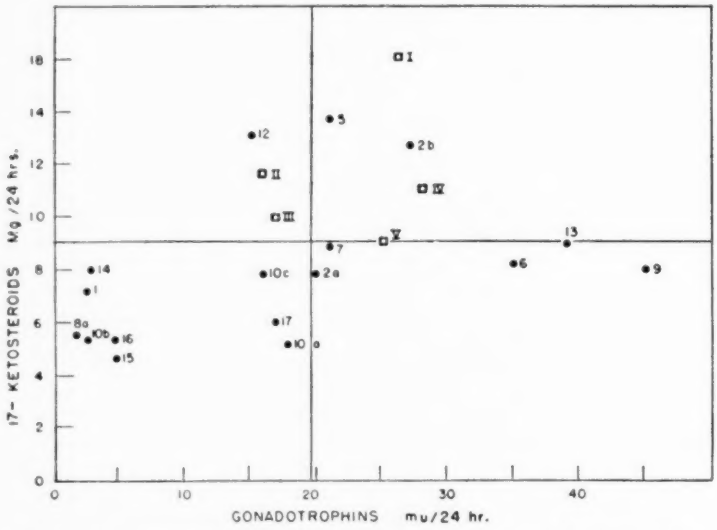

FIG. 7. THE RELATIONSHIP OF URINARY 17-KETOSTEROIDS AND GONADOTROPHINS

The numbers indicate the patient and period number (see Table I). Normal controls are indicated by Roman numerals. The crossed lines indicate the mean 17-ketosteroid and gonadotrophin values for the group. Rank correlation analysis indicated a correlation that was not significant at the $P = 0.05$ level but was at the $P = 0.1$ level.

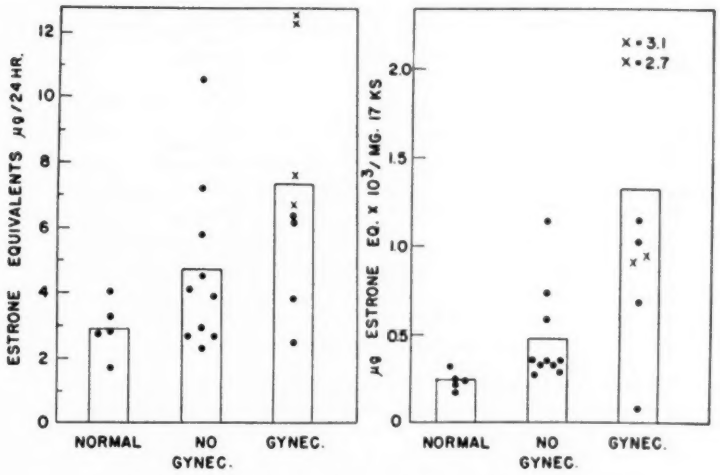

FIG. 8. THE RELATIONSHIP OF THE URINARY EXCRETION OF TOTAL ESTROGENS AND THE ESTROGEN/17-KETOSTEROID RATIO TO GYNECOMASTIA IN MEN WITH LIVER DISEASE

The first column in each section indicates the values in normal men, the second column that of men with liver disease without gynecomastia, and the third column men with liver disease and gynecomastia. In the third column, the symbol × indicates individuals with stable or advancing gynecomastia, and ● in the same column indicates patients with gynecomastia which disappeared within four months. Patient No. 2 with most marked gynecomastia, excreted 7.6 µg. of estrone equivalent per 24 hr. during period "a." Striking improvement occurred during aureomycin therapy. Six months later, the gynecomastia had disappeared. Urine excretion value at this time (period "b") was 4.6 µg./24 hr. and is included in the group with no gynecomastia. In the two other cases with multiple observations, the average values were used since there was no change in classification. In the three other patients with "stable or advancing gynecomastia" one patient (No. 3) continued to have three plus gynecomastia for at least seven months, another (No. 8) showed increasing gynecomastia between his two periods of urine collection. The third patient (No. 15) died four weeks after the observation was made.

patients with stable (or advancing) "skin spiders" when compared to that of the four patients without them or to the five normal controls as shown in Figure 9. The differences in the total free form and in the other fractions are not significant. Patient No. 7 had the most "skin spiders" and many of these showed arterial pulsations. This patient exhibited the highest excretion of the free form of all three fractions.

Palmar erythema. The three patients with palmar erythema did not exhibit significant variation in free or total estrogens, the estrogen fractions or 17-ketosteroid excretion from patients without this symptom. The gonadotrophin value in the one patient tested was normal.

Testicular atrophy and loss of libido. Figure 10 shows the relationship of the urinary hormone excretion to the presence of definite testicular atrophy (with loss of libido) and loss of libido (without detected testicular atrophy). A highly significant deviation from the normal values for gonadotrophin, 17-ketosteroids and estrogens is apparent for those men with loss of libido or testicular atrophy. A highly significant reduction in the gonadotrophic hormone excretion, and a decrease in total neutral 17-ketosteroid excretion of borderline significance, are found when this group is compared to patients without these symptoms. No significant differences between these two groups are found for total estrogens or

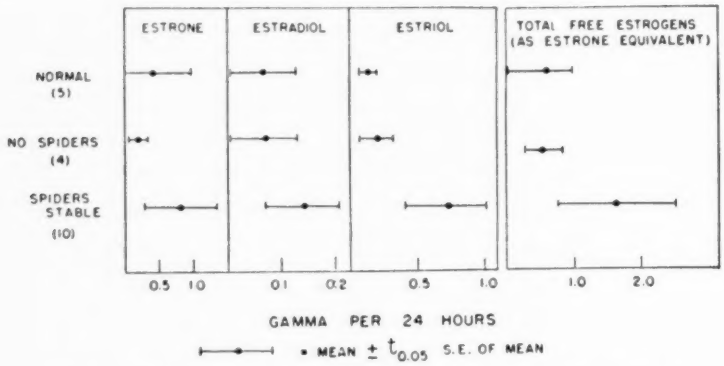

FIG. 9. CUTANEOUS SPIDER NEVI AND UNCONJUGATED ESTROGEN FRACTIONS

The free "estriol" fraction was significantly greater in men with stable (or advancing) "skin spiders" than in normal individuals and patients without them. Three patients (values not charted) in whom "skin spiders" were present, but regressing, had approximately the same urinary excretion of free "estriol" as the patients without these lesions.

the free and conjugated forms of the "estrone," "estradiol," and "estriol" fractions (not shown in Figure 10). That age may be an important factor in these relationships is indicated by the average age of 33.3 years (range 22 to 46) in those without testicular atrophy or loss of libido; while those with one or both of these symptoms had an average age of 47.7 years ranging from

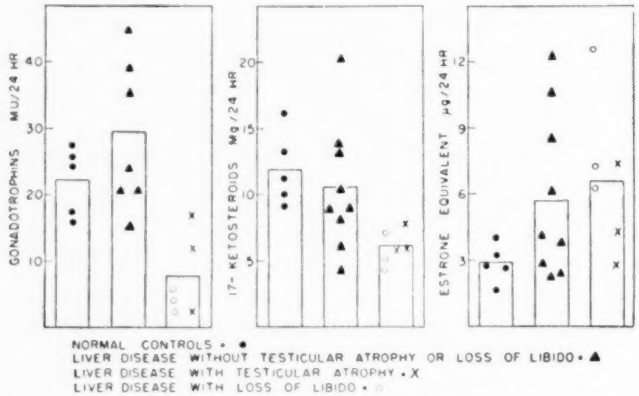

FIG. 10. HORMONE EXCRETION AND TESTICULAR ATROPHY AND LOSS OF LIBIDO

In addition to the three patients with evident testicular atrophy and complete loss of libido, three other patients also had complete loss of libido. In two, scrotal edema prevented adequate estimation of testicular size and in the third, testes slightly smaller than the lower limits of normal were measured. These six patients have been included in a group, but differentiated by symbols as indicated. Three patients had analyses for more than one period. The data are pooled for each individual since there was no change in classification.

35 to 60 years. In addition, men with these symptoms were, in general, more severely ill (grade III and IV) than those men without them.

Miscellaneous observations

The total neutral ketonic and non-ketonic fractions were determined on the ether extracts of the urine from five normal men and seven men with severe liver disease. The non-ketonic fractions were essentially the same for both groups while the mean total ketonic fraction for the men with liver disease was slightly less than one-half of that for normal men. This result is to be expected since the urinary excretion of 17-ketosteroids is depressed in severe liver disease.

The "free" fraction of neutral 17-ketosteroids was determined in 37 men with diffuse liver disease and five normal men. The difference between the means of the free fraction of the two groups was not significant, nor was it significant if only those patients with grade III or IV liver disease were compared with the normal individuals. These findings are similar to those of Williams, Cantarow, Paschkis, and Havens (*22*). These authors have also presented data indicating some decreased ability to conjugate the increased amount of 17-ketosteroids produced by injection of testosterone propionate.

The urinary excretion of reducing corticoids was examined for periods of one to five days in 11 men and three women with varying degrees of diffuse liver disease. Seven of the 14 individuals had one or more 24 hour specimens with corticoid values greater than those for normal controls. Similar findings have been reported by Bongiovanni and Eisenmenger (*23*). The significance is unknown.

DISCUSSION

Hormone excretion and severity of liver disease

Clinical and pathological data have provided evidence in many patients with liver disease that there was probably an increase in the effective concentration of estrogens in the body fluids. The pioneer work of Glass, Edmondson, and Soll (*2*) afforded support for this concept by the finding of increased excretion of estrogens in the urine. Our findings, as well as those of Rupp, Cantarow, Rakoff, and Paschkis (*24*) and Humm, Munson, and Salter (*25*) and others indicate an increase in total estrogen levels and decreased levels of neutral 17-ketosteroids in some patients with chronic liver disease, in so far as blood levels can be inferred from urinary excretion values. Although the most severe grade of liver disease was associated with the highest total estrogen and the lowest 17-ketosteroid excretion, the relationship of urinary excretion of these substances to the severity of the disease, as judged by our criteria, was not clear-cut (see Table I). Somewhat smaller increases in total estrogens and decreases in 17-ketosteroids have also been found in the acute stages of hepatitis, subsiding as the patient improved (*26*). The effect of liver disease on the excretion of unconjugated estrogens and estrogen fractions is discussed below.

Correlation of total estrogen excretion with liver function

Figure 1 shows some mechanisms possibly involved in ridding the body of active estrogens. Increased production of estrogens seems improbable as an explanation for the estrogenic effects noted in some patients with liver disease, but remains a possibility. Impaired excretion or destruction, or both, seems to be a more probable explanation. The effect of conjugation on activity is discussed below. Figures 2 and 3 demonstrate the positive linear correlation of urinary estrogen excretion with serum bilirubin and bromsulphalein retention over a limited range. Since these tests are considered to be a measure of "excretory function" of the liver, and since there is no significant correlation of estrogen excretion with other liver function tests nor as distinct a correlation with the general severity of the disease, it seems probable that the increased urinary excretion is, in part, due to decreased excretion through the biliary tract or to a decrease of some other functions of the liver reflected by these tests. It is important to note that this relationship is demonstrated only for patients with lesser degrees of jaundice and was not present in two patients with severe jaundice. Decreased production of estrogen in these severely ill men is suggested as a probable explanation for this finding.

The biliary excretion of estrogens and their

esters has been recently reviewed (3, 10). Biliary excretion has been demonstrated in laboratory animals and in man by bio-assay and chemical methods. Estrogenic activity has been found in the feces of normal men in amounts up to seven times that found in the urine (27, 28). In contrast, Gallagher, Fukushima, Barry, and Dobriner (29) have failed to find free or conjugated 17-ketosteroids in feces of normal men. Recent work using estrogens labeled with radioactive halogens has shown that a high proportion of such compounds are excreted by way of the bile duct in both the mouse (10) and man (30). The quantities of radioactive material found in feces are considerably greater than would be expected from bio-assays and suggest that selective excretion of the halogenated compounds may have occurred or that inactive or unextracted estrogens are present in the feces (10). Thus, it seems possible that failure of excretion of estrogens through the biliary tract may account, in part, for the increased urinary excretion found in some patients with liver disease.

Excretion of estrogen fractions and the free and conjugated forms

In this discussion of the "estrone," "estradiol," and "estriol" fractions, it must be remembered that separation of these three steroids is not absolute even by the best partition methods (9, 10). Our single experiment (described in Methods) would support this conclusion; especially for the small amounts of estrogens found in male urine. Nevertheless, this and the partition studies of others (9, 10) make it clear that the major activity of a fraction may be properly ascribed. It will be noted that our values for the estrogen fractions in the normal men are less than those reported by Pincus (31). This is presumably due to difference in preservation, hydrolysis, and extraction technics.

In the men with liver disease, the major increase occurred in the free and conjugated form of the estriol fraction. It seems unlikely that hepatic disease would be associated with increased conversion of estradiol and estrone to estriol. In fact, there is some evidence from studies on a man with cirrhosis of decreased conversion to estriol (32). Since nothing is known of the type of estrogen produced by the adrenal and testes of the human male, the significance of this finding is not clear.

Only six of the 17 patients in our series showed an increase in the unconjugated estrogens. This agrees with the finding of Rupp and associates (24), but is a smaller proportion than that found by Glass, Edmondson, and Soll (2). During the past 10 years, it has become abundantly evident that hydrolysis of steroid conjugates may occur if urine is allowed to remain at room temperature, and even to some extent at 4° C. Therefore, this possibility must be considered in any study involving analysis of the free form. We believe that our findings represent a true difference in values between patients with liver disease and the normal controls, since the urine specimens were handled in a similar fashion for both groups, care being taken to decrease the possibility of spontaneous hydrolysis.

In discussing the physiological role of the free form of estrogens and of estriol, it seems proper to reconsider the concept that the conjugated steroids and estriol are considerably "less active" (8). These ideas were based on data primarily derived from assays employing subcutaneous or intramuscular injections. Differences in rates of absorption from the site of injection is a recognized factor in the effect of the various forms of estrogen on the target tissues and for this reason this technic does not measure the true potency at the level of the target tissue. Intravaginal assays are believed to afford a better comparison of potency at the tissue level. Robson and Adler have demonstrated the local effectiveness of estriol glucuronide by instilling it into one section of the surgically made double vagina in mice (6). Furthermore, Emmens' (7) assays of certain esterified and non-esterified estrogens, by intravaginal technic show but little, if any, difference in activity of the two forms. The intravaginal assay of estrogens absorbed on various proteins demonstrates that estradiol is only slightly more active than estrone and estriol, which have the same potency (33). However, we are unaware of comparative assays of the free form and the glucuronide of estrone, estradiol, and estriol done by the multiple dose intravaginal technic. Since many tissues in the body show glucuronidase activity,

it seems probable that conjugation with glucuronic acid or lack of it is not an important factor in determining estrogenic activity for such tissues (34). Therefore, it is believed that the production of the conjugated forms and of estriol from estrone or estradiol, should no longer be considered important mechanisms for decreasing the activity of estrogens. Furthermore, we have not found a significant correlation of any physiological effect of estrogen with the urinary excretion of the total free fraction. The relationship of the free estriol fraction to the presence of cutaneous spider nevi is discussed later.

Inter-relationships in urinary excretion of hormones

The relationship of urinary 17-ketosteroid and gonadotrophin excretion (Figure 7) in these patients is supporting evidence of the role of gonadotrophic hormones in the production of 17-ketosteroids in males. It seems probable that the large proportion of urinary 17-ketosteroid formed from adrenal cortical steroids partially obscures this relationship. The relatively lower gonadotrophin excretion with high excretion levels of estrogen, as demonstrated in Figure 5, is also to be expected, since there is considerable physiological data supporting the concept that increased levels of blood estrogen are associated with decreased gonadotrophin secretion. It is of interest that relatively slight increases of estrogen excretion are associated with apparent decrease in gonadotrophin excretion and there are apparently even more definite relationships with 17-ketosteroid excretion (Figure 6).

Correlation of hormone excretion with clinical manifestations

The increased excretion of total estrogens and decrease in 17-ketosteroids in association with stable or advancing gynecomastia in men with liver disease needs but little comment. The apparent lack of correlation in some investigations (24, 35) may, in part, be due to regressing gynecomastia being included in the series. In four of our eight cases of gynecomastia, this symptom disappeared within four months of the urine collection period. In two of these men, the estrogen excretion was normal, and in two, it was only moderately elevated (Figure 8). Thus, observations on hormone-tissue relationships may be confusing unless the condition of the target tissue at the time of the urine collection is known. There was no relationship between the free form of estrogen and the presence of gynecomastia. This does not support the thesis of Glass, Edmondson, and Soll (2) that gynecomastia is due to an increase in the free form of estrogens.

The possibility exists that the tissue androgen level may play a role in the production of gynecomastia. It has been demonstrated by Mühlbock (36) that testosterone may inhibit the growth of breast tissue, ordinarily produced by estrone in the castrate male rat. Recent observations in two patients with adrenal cortical tumors secreting large amounts of estrogen and androgens without gynecomastia and in two patients with adrenal tumors and exhibiting gynecomastia and high urinary estrogen values with normal 17-ketosteroids, suggest that this may also be true, to some extent, in man (37). With this in view, the estrogen-ketosteroid ratio was calculated and charted in Figure 8. It will be noted that there was a greater difference between those patients having gynecomastia and liver disease, and those with liver disease but without gynecomastia when charted in this fashion, than when these groups are compared on the basis of total estrogen excretion. The appearance of gynecomastia during testosterone administration, and many other unexplained observations (38) serve to emphasize the difficulties in the over-simplified considerations detailed above.

There was no correlation between the presence of cutaneous spider nevi and the urinary excretion of total estrogens. However, clinical evidence (39) strongly supports some role of estrogens in the production of cutaneous spider nevi, but the peculiar distribution of "skin spiders" and other considerations indicate that other factors than estrogens are probably of importance in the production of these lesions. Our data show a significant correlation between the presence of non-regressing "skin spiders" and the urinary excretion of estriol in the free form. This surprising finding suggests that the free form of estriol may stimulate growth of certain blood vessels under special conditions. It also suggests

the absence of glucuronidase activity in the blood vessels of the skin.

Testicular atrophy and loss of libido in patients with liver disease is associated with significantly lower values of urinary gonadotrophins and a decrease of borderline statistical significance in the 17-ketosteroids. Although the method employed to measure "gonadotrophins" in this study is believed (18) to measure primarily the effect of follicle stimulating hormone (FSH), it is probable that the presence of some interstitial cell stimulating hormone (ICSH) is necessary for activity. Thus, suggestive evidence is afforded that the decreased 17-ketosteroid excretion in these men is due, in part, to decreased production of androgens by the Leydig cells of the testes as a result of decreased gonadotrophic hormone secretion by the pituitary. The effect of chronic illness on adrenal cortical function must also be considered. The decreased androgens may, in this instance, be an important factor in loss of libido. The stimulating effect of intramuscular injection of testosterone upon sexual desire in men with liver disease has been briefly noted by Rosenak, Moser, and Kilgore (40). The testicular atrophy may be attributed to the decrease in gonadotrophins. The relative importance of FSH and androgens in support of the tubular tissue of the adult human testis is obscure. From inspection of the data, it seems likely that the increase in circulating estrogens (presumed from the urinary excretion values) is not the only factor responsible for inhibition of gonadotrophin production. Age and impaired nutrition may play a role.

SUMMARY

The urinary excretion of the unconjugated and conjugated forms of the "estrone," estradiol," and "estriol" fractions, as well as neutral 17-ketosteroids and gonadotrophins, has been determined for one or more three to 22 day periods in five normal men and 17 men with diffuse hepatic disease. The results support the concept of disturbed hormone metabolism as a cause for some of the symptoms of diffuse hepatic disease and suggest that decreased biliary excretion may, in part, be responsible for the increased urinary excretion of estrogens. The main findings are listed below:

Estrogens. (*1*) Total estrogen excretion was increased in 11, and the unconjugated form in six of the patients. The group with most severe liver disease exhibited the highest excretion of both conjugated and unconjugated forms of estrogens. (*2*) The excretion of total estrogens showed a highly significant correlation with bromsulphalein retention and the total serum bilirubin values in patients with mild jaundice. Two patients with severe jaundice did not show increased estrogen excretion. There was no significant correlation with other tests of liver function. (*3*) The excretion of total estrogens (but not that of the unconjugated form) was significantly increased in the four patients with advancing or stable gynecomastia in comparison to that of patients with regressing or no gynecomastia. (*4*) In general, a high total estrogen excretion was associated with less than average gonadotrophin and 17-ketosteroid excretion. (*5*) Both the conjugated and unconjugated forms of "estriol" were increased to a greater extent and more frequently than "estrone" or "estradiol." (*6*) The excretion of unconjugated "estriol" was significantly increased in the patients exhibiting stable or advancing cutaneous spider nevi.

17-Ketosteroids. The 17-ketosteroid excretion was less than normal in 14 of the 17 patients and was decreased in the four men with stable or advancing gynecomastia and the six men with testicular atrophy and/or loss of libido when compared to patients without these symptoms.

Gonadotrophins. Gonadotrophins were significantly decreased in the six patients with testicular atrophy and/or complete loss of libido.

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THE EFFECT OF EPINEPHRINE (USP), *l*-EPINEPHRINE, AND *l*-NOREPINEPHRINE ON GLOMERULAR FILTRATION RATE, RENAL PLASMA FLOW, AND THE URINARY EXCRETION OF SODIUM, POTASSIUM, AND WATER IN NORMAL MAN [1, 2]

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The adrenal medulla apparently elaborates at least two highly active pressor principles, *l*-epinephrine and *l*-norepinephrine, that may profoundly affect the renal circulation in man. In physiologic dosage both substances evoke an intrarenal vasoconstriction which tends to reduce renal blood flow in the face of an elevated arterial pressure without significant alteration in glomerular filtration rate (1–3). Associated changes in urine formation might be predicted but have not as yet been clearly defined. In animals, *l*-epinephrine seems to give rise to a water diuresis and augmented excretion of chloride (4–8), whereas in man both agents may diminish urine flow (1, 2). A study of the effect of pure *l*-epinephrine and *l*-norepinephrine upon renal hemodynamics, and excretion of water and electrolytes in normal human subjects was undertaken in order to clarify this problem. Since most of the earlier work is based upon the use of adrenal extracts which have been found to contain both pressor amines (9, 10), the effect of epinephrine (USP) was also examined in order to ascertain whether its action is specific or predominantly that of one of its ingredients.

METHODS

The subjects of this study were 17 convalescent patients (seven male, ten female) from the wards of the Presbyterian Hospital in the City of New York. All were free of clinically demonstrable cardiac and renal disease. They received a well-balanced hospital diet containing approximately 10 grams of sodium chloride each day. The effects of *l*-norepinephrine [5] were determined on 12 occasions, and of *l*-epinephrine [6] and epinephrine (USP) three times each. Glomerular filtration was measured as the inulin clearance, and renal plasma flow as the sodium *p*-aminohippurate clearance.[7] The renal clearances of sodium and potassium were determined simultaneously. The blood pressure was measured sphygmomanometrically, and mean pressure calculated after the method of Böger and Wezler (11) for use in computation of renal vascular resistance. The methods employed in the experimental procedures and chemical analyses have been described at length elsewhere (12, 13). All solutions were made up in 5% dextrose and distilled water. Three successive resting control clearance values were determined at ten-minute intervals prior to intravenous administration of an infusion of the pressor agent under study (4 to 8 μg./ml. of 5% dextrose in distilled water) at a rate which was maintained constant at about 15 μg./min. in most individuals. In one very susceptible subject the rate of infusion (*l*-epinephrine) could not be raised above 2 μg./min. without discomfort, and in another 46 μg./min. of *l*-norepinephrine was required to produce the desired effect. An effort was made to maintain the mean arterial tension between 25 and 50 mm. Hg above the control without undue subjective reaction. These precautions eliminated the possible role of fright or discomfort in producing the observed effects. The urine voided during the period of equilibration of the arterial pressure at a constant level was discarded and three successive determinations were then made over an interval of from 30 to 45 minutes. In ten instances, two more clearance values were obtained after the blood pressure had returned to control levels and after a 15-minute discard period to permit equilibration.

RESULTS

Systemic circulatory adjustments and subjective response. The systemic hemodynamic and

[1] This work has been aided by a grant from the American Heart Association.

[2] These data were presented in detail before the International Congress of Clinical Pathology, London, England, on July 19, 1951 (33).

[3] Research Fellow of the American College of Physicians.

[4] Fellow of the New York Heart Association.

[5] Supplied through the courtesy of the Medical Research Department, Winthrop-Stearns, Inc., as "Levophed."

[6] We are indebted to Dr. Maurice Rapport for a supply of pure *l*-epinephrine.

[7] We are indebted to Dr. W. Boger and the Medical Research Division of Sharp and Dohme, Inc., Philadelphia, Pa., for a generous supply of sodium *p*-aminohippurate.

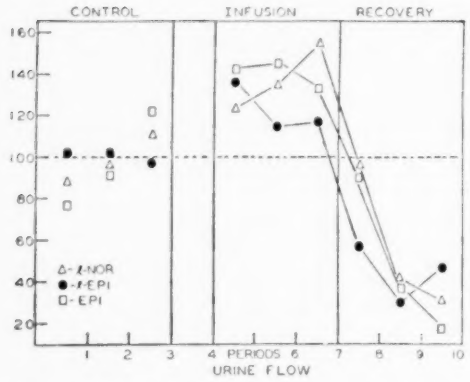

FIG. 1. THE EFFECT OF EPINEPHRINE (USP), *l*-EPINEPHRINE AND *l*-NOREPINEPHRINE ON URINE FLOW

The values for urine flow during each "period" (usually ten to 15 minutes in length) have been averaged and expressed here as the percentage of the pooled and averaged control figures. An infusion of epinephrine (USP) (open squares), *l*-epinephrine (closed circles), or *l*-norepinephrine (open triangles) was administered intravenously during the periods marked "Infusion." With one exception (P. D.) in Table I, in whom only periods 8 and 9 were obtained during recovery, all data presented here were collected in complete experiments. Three experiments are thus averaged together for each substance, *viz.*, epinephrine (USP)—H. J., C. N., and G. G.; *l*-epinephrine—A. B., M. W. and G. I.; *l*-norepinephrine—I. C., A. B., and E. R. (See Table I for further information regarding these subjects.) The urine flow rose slightly during the infusion of all the pressor amines and fell sharply on withdrawal during the "Recovery" periods.

subjective responses to epinephrine (USP), *l*-epinephrine and *l*-norepinephrine observed during the course of this study were similar to those reported previously (13–15). Epinephrine (USP) and *l*-epinephrine had a similar effect in producing an elevation in systolic pressure with slight or no change in diastolic pressure. On the average, systolic pressure increased 20 and 35 mm. Hg and diastolic increased 4 and 8 mm. Hg, respectively, in association with tachycardia. Despite these relatively slight changes in arterial tension, the subjects usually complained of nervousness and palpitation, the heart rate was occasionally irregular (premature contractions) and tremor and pallor were often striking. On termination of the infusion the skin became flushed and a sensation of increased body warmth was experienced. In contrast, *l*-norepinephrine in approximately equal dosage brought about a more striking elevation in both systolic (averaging 50 mm. Hg) and diastolic (25 mm. Hg) pressures in association with a marked bradycardia and very little subjective disturbance. Some subjects complained of slight headache, dizziness, substernal oppression and breathlessness; others suffered no discomfort whatever. Hyperemia of the skin was not noticeable following withdrawal of the drug.

Renal vascular adjustments. Despite these differences in systemic hemodynamic activity all three substances affected renal function similarly. The averaged figures for renal plasma flow (RPF) and glomerular filtration rate (GFR) during the control period, during the infusion of the pressor agent and during the recovery period are presented in Table I. It can be seen that renal plasma flow (RPF) decreased in all but one (H. J.), in whom *l*-epinephrine produced renal vasoconstriction proportionate to the elevation in arterial pressure so that there was no change in RPF. Glomerular filtration (GFR) remained relatively unchanged or increased slightly so that the filtration fraction (GFR/RPF) always rose. The calculated renal vascular resistance was invariably increased—to a much greater extent by *l*-norepinephrine than by either *l*-epinephrine or epinephrine (USP). These changes were rapidly reversible on termination of the infusion. A moderate increase in renal blood flow was observed approximately 30 minutes following epinephrine (USP) and *l*-epinephrine that did not develop following *l*-norepinephrine.

Changes in water and electrolyte output. All three agents influenced the urinary excretion of sodium, potassium, and water in the same way. The plasma sodium and potassium concentration did not change significantly at any time. Sodium and potassium output in the urine usually decreased sharply and significantly [8] whereas urine

[8] Statistical analysis indicates that these changes are highly significant. Although electrolyte output varied widely from subject to subject, the variation within any single individual was relatively small during periods of time equivalent to those covered in these experiments. Similar studies of inactive agents, such as small doses of isotopes, revealed no changes in electrolyte excretion greater than those occurring spontaneously during the control periods. Hence, the observations reported here cannot be accounted for on the basis of chance variations or of nonspecific effects of the experimental procedure.

flow showed no consistent directional change. On termination of the infusion and on the return of blood pressure to normal values, sodium and potassium excretion tended to return toward control levels and urine flow usually fell off abruptly (Table I and Figures 1 to 3). Urine flow (V) increased markedly in two of the three patients receiving epinephrine (USP) and in all three fell off to or below the control level during recovery. The urine flow increased (Table I) significantly in only one of three studies with *l*-epinephrine and decreased sharply during recovery in all three. The diuretic tendency was much less evident during the action of *l*-norepinephrine. Averaged urine flows were observed to rise in three (P. D., T. G., F. E.), remain relatively unchanged in six, and fall in three (D. C., K. N., P. M.). In two of four measurements made during recovery the urine flow diminished. Since many of these patients were responding by diuresis to water ingested prior to the study, the rise in urine flow may have been coincidental. In Figure 1, the values for urine flow during each clearance measurement have been averaged and plotted as the percentage of the total control mean values, using only those experiments in which recovery was followed for at least 30 minutes. The discard period excluded from the averaged recovery values in Table I have been included in this figure. The similarity of the response to *l*-norepinephrine, *l*-epinephrine, and epinephrine (USP) is obvious. This figure brings out more clearly than the averages presented in Table I the tendency for urine flow to fall below the control values during recovery from the pressor effect, and indicates the lack of clear-cut evidence for the development of water diuresis during pressor activity.

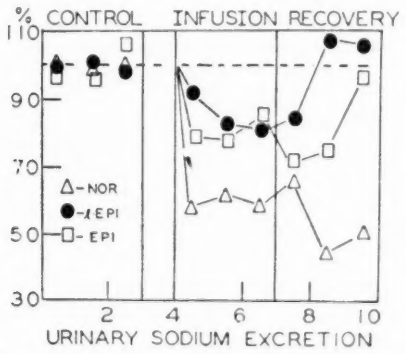

FIG. 2. THE EFFECT OF EPINEPHRINE (USP), *l*-EPINEPHRINE, AND *l*-NOREPINEPHRINE ON URINARY SODIUM EXCRETION

The data for urinary sodium excretion in the subjects presented in Figure 1 are handled here in the same manner. Sodium excretion decreased during the drug action and tended to return to the control levels following termination of the infusion.

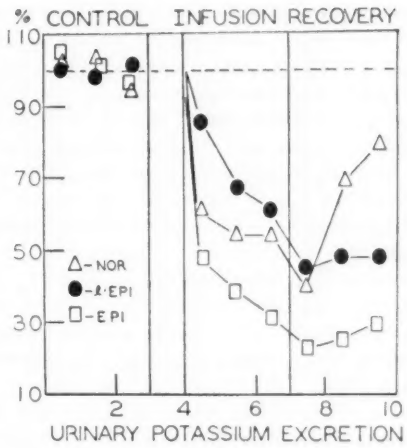

FIG. 3. THE EFFECT OF EPINEPHRINE (USP), *l*-EPINEPHRINE, AND *l*-NOREPINEPHRINE ON URINARY POTASSIUM EXCRETION

The data are treated as in Figure 1. Potassium excretion decreased markedly during infusion of each drug and tended to remain low thereafter.

Sodium excretion (Una V in Table I) decreased on the average during the infusion of epinephrine (USP) and *l*-epinephrine in two or three studies each, and in nine of 11 during infusion of *l*-norepinephrine. During the recovery period sodium excretion returned to or above the control in one subject after epinephrine (USP) and in two after *l*-epinephrine. It did not reach the control level in any of the three followed during recovery from the action of *l*-norepinephrine. These effects are more obvious in Figure 2 which is constructed like Figure 1, on the basis of averaged values for each clearance period, including the recovery discard period expressed in terms of the total control mean. It can be seen that *l*-norepinephrine appeared to have a much

more profound effect in depressing sodium excretion than either *l*-epinephrine or epinephrine (USP). As a rule sodium excretion fell off much more than urine flow and the urinary concentration of sodium diminished (Table I). Sodium excretion decreased more than the filtration rate so that the sodium-inulin clearance ratio (Cna/Cin in Table I) fell in all but P. M. and E. B. (*l*-norepinephrine), indicating augmented tubular reabsorption of sodium.

Potassium excretion was also depressed by the action of epinephrine (USP), *l*-epinephrine, and *l*-norepinephrine. This change occurred without exception and persisted throughout the recovery period (Table I and Figure 3). The urinary potassium concentration always decreased and the potassium-inulin clearance ratio (Ck/Cin in Table I) fell and remained low following recovery. Hence, the tubular reabsorption of potassium was always augmented by the adrenal medullary hormones. In general, *l*-norepinephrine appeared to produce a more profound change in sodium excretion than in potassium excretion whereas *l*-epinephrine had a greater effect on the output of potassium.

DISCUSSION

Despite a noteworthy divergence in systemic hemodynamic activity, *l*-norepinephrine and *l*-epinephrine produce more or less identical changes in renal function. Epinephrine (USP), a mixture of the two, behaves like *l*-epinephrine in its action upon the circulation and the kidney. Overall vasodilatation is manifest in elevated cardiac output with little or no change in mean arterial pressure during the action of *l*-epinephrine and epinephrine (USP), whereas generalized vasoconstriction is evident in diminished cardiac output and elevated arterial pressure during the response to *l*-norepinephrine (3, 14, 15). All excite a renal vasoconstriction that reduces renal blood flow without significant change in filtration (1–3). The present study has revealed further that all appear to interfere to some extent with tubular reabsorption of water and that all give rise to augmented tubular reabsorption of sodium

TABLE I

The effect of adrenal medullary hormones on renal function *

Sex	Subject Age	S.A. M^2	Procedure	B.P. *mm.Hg*	P.R. *per min.*	V *ml./min.*	GFR *ml./min.*	RPF *ml./min.*	FF %	Una V *μEq./min.*	Uk V *μEq./min.*	Cna/Cin %	Ck/Cin %
						epinephrine (USP)							
M	H. J. 49	1.55	Control	118/80	72	1.6	90	470	19	74.6	45.3	0.59	12.96
			Infusion	138/80	80	6.1	95	480	20	55.2	21.0	0.42	5.65
			Recovery	122/72	74	1.1	110	555	20	89.2	20.6	0.56	4.72
F	C. N. 37	1.67	Control	100/64	54	6.3	105	500	21	103.1	63.3	0.72	13.74
			Infusion	130/70	75	8.0	105	375	28	97.6	18.3	0.68	3.94
			Recovery	98/58	60	0.8	95	520	18	92.1	11.7	0.72	2.83
M	G. G. 39	1.95	Control	120/70	70	12.2	130	680	19	373.6	145.4	2.04	24.28
			Infusion	138/74	85	13.3	145	550	27	277.8	65.6	1.37	10.10
			Recovery	124/64	74	1.5	130	740	18	202.2	27.9	1.14	4.74
						l-epinephrine							
M	A. B. 44	1.64	Control	120/84	95	0.9	105	440	24	86.5	50.8	0.63	10.00
			Infusion	148/84	110	0.8	105	395	27	81.0	33.1	0.59	7.19
			Recovery	128/84	105	1.0	115	455	25	118.0	23.6	0.79	4.27
F	M. W. 26	1.58	Control	100/74	76	5.5	110	540	21	118.4	21.4	0.81	5.23
			Infusion	140/82	90	9.1	95	340	28	90.5	16.1	0.71	4.50
			Recovery	100/62	90	1.8	115	560	20	123.0	14.4	0.83	3.40
F	G. I. 29	1.53	Control	114/76	72	7.8	100	600	17	78.6	85.3	0.58	19.70
			Infusion*	150/90	94	6.2	115	495	24	63.8	57.3	0.41	11.36
			Recovery	118/50	74	2.8	125	745	17	61.4	26.2	0.36	4.87

TABLE I—*Continued*

Sex	Subject Age	S.A. M^2	Procedure	B.P. *mm.Hg*	P.R. *per min.*	V *ml./min.*	GFR *ml./min.*	RPF *ml./min.*	FF %	Una V *μEq./min.*	Uk V *μEq./min.*	Cna/Cin %	Ck/Cin %
						l-norepinephrine							
F	I. C. 23	1.56	Control	100/62	70	7.9	145	665	22	176.4	50.7	0.87	8.04
			Infusion	150/90	56	8.9	155	465	33	54.0	32.7	0.25	5.00
			Recovery	106/60	70	0.8	155	550	26	74.4	34.5	0.34	5.15
M	A. B. 44	1.64	Control	124/88	94	1.3	105	425	24	104.3	65.6	0.75	12.03
			Infusion	172/108	90	1.2	75	270	28	28.3	28.3	0.29	7.21
			Recovery	124/90	105	2.5	105	440	24	61.6	52.0	0.44	9.34
F	E. R. 22	1.32	Control	88/54	104	2.7	85	340	25	40.1	60.5	0.37	15.22
			Infusion	132/94	75	2.0	55	125	44	11.4	16.2	0.15	8.33
			Recovery*	98/56	74	2.4	85	365	23	26.4	39.1	0.24	13.45
M	P. D. 47	1.89	Control	120/80	66	7.4	140	710	20	—	—	—	—
			Infusion	170/102	—	12.7	135	560	24	—	—	—	—
			Recovery	110/74	68	2.8	95	500	19	—	—	—	—
M	L. F. 30	1.93	Control	108/80	64	7.1	90	520	18	96.7	92.9	0.72	25.30
			Infusion	158/106	60	6.3	120	485	24	47.1	78.2	0.29	14.60
M	H. P. 52	1.74	Control	112/70	75	9.0	85	390	22	161.2	55.2	1.38	13.20
			Infusion	164/90	50	9.3	90	315	29	140.9	36.2	1.12	8.03
F	D. C. 18	1.55	Control	94/54	72	5.8	90	535	17	118.1	63.2	0.91	18.10
			Infusion	166/100	60	4.1	95	295	32	67.2	23.3	0.50	6.33
M	T. G. 50	1.85	Control	110/72	60	2.4	115	650	19	115.1	104.0	0.77	21.19
			Infusion	168/90	52	10.5	110	435	25	104.0	77.3	0.52	16.80
F	K. N. 30	1.46	Control	122/62	96	13.2	105	540	19	93.6	52.8	0.65	12.78
			Infusion	146/82	68	8.4	90	325	28	40.2	32.7	0.31	9.29
F	F. E. 34	1.90	Control	112/74	85	6.2	115	575	20	101.0	81.2	0.64	17.86
			Infusion	146/88	78	8.5	110	430	25	80.0	49.1	0.54	11.22
F	P. M. 28	1.87	Control	94/60	65	11.9	105	630	17	164.2	130.0	1.11	27.20
			Infusion	144/88	55	9.7	105	500	22	169.3	83.6	1.27	17.20
F	E. B. 25	1.72	Control	102/66	80	8.6	130	535	24	51.5	37.3	0.30	6.01
			Infusion	138/92	65	8.8	135	495	28	73.4	29.5	0.40	4.47

* All values are averages of two or more determinations except in instances marked with an asterisk. Infusions of epinephrine (USP), *l*-epinephrine, or *l*-norepinephrine were administered throughout "infusion" period at a rate sufficient to produce an elevation in arterial pressure without undue discomfort. The arterial pressure (B.P.) was measured sphygmomanometrically. Abbreviations are as follows:

S. A. = body surface (M^2)
P. R. = pulse rate
V = urine flow (ml./min.)
GFR = glomerular filtration rate, inulin clearance (ml./min.)
RPF = renal plasma flow, sodium p-aminohippurate clearance (ml./min.)
FF = filtration fraction—GFR/RPF (%)
Una V = urinary sodium output (μEq./min.)
Uk V = urinary potassium output (μEq./min.)
Cna/Cin = sodium-inulin clearance ratio or proportion of filtered sodium excreted (%)
Ck/Cin = potassium-inulin clearance ratio or proportion of filtered potassium excreted (%)

and potassium. Since maximal tubular reabsorptive (glucose Tm) and excretory (diodrast Tm) capacities are not affected by epinephrine (USP) (16), it is not likely that either *l*-epinephrine or *l*-norepinephrine have any effect on Tm. It may be inferred from this and much additional direct evidence (17–19) that the renal vasculature and parenchyma are diffusely and totally involved in the observed response.

The mechanism of the change in urine formation is not yet apparent. Since it appears when blood pressure is little altered or, contrariwise.

greatly increased, when tachycardia indicates increased cardiac output during the action of *l*-epinephrine, or when bradycardia denotes diminished output with *l*-norepinephrine, it may be presumed that the general systemic hemodynamic adjustment is not a determining factor. Attention must be paid rather to the local renal circulatory adjustments and to the possibility of direct or indirect influence upon tubular cellular activity by the drugs themselves.

The site of intrarenal vasoconstriction has been placed by Smith and others in the post-glomerular vascular bed. This view is based upon the observations of Richards and Plant (*20, 21*) that perfused kidneys may swell during the action of adrenal extracts (and presumably also *l*-epinephrine), and of Smith and his associates (*1, 22*) that glomerular filtration does not decrease. The first is interpreted as evidence for engorgement of a sizeable portion of the renal vascular bed proximal to the point of constriction. Since filtration does not increase it may be presumed that intraglomerular filtration pressure must rise in proportion to the decrement in renal plasma flow independently of any alteration in systemic arterial pressure. In this view afferent arteriolar constriction develops in proportion to the elevation in the blood pressure and vasoconstriction in the post-glomerular vascular bed accounts for depression of renal blood flow below the control value. Gomez (*22, 23*) has placed the site of this activity in the venular bed in order to account for the swelling of perfused kidneys. From this it follows that intrarenal pressure may rise and bring about the observed augmentation in tubular reabsorption of sodium. But studies (*24*) of the effect of increased intra-abdominal pressure, that presumably increases intrarenal tension, indicate that water reabsorption is also increased and that potassium reabsorption is relatively unaffected. Hence, it seems unlikely that increased interstitial pressure in the kidney was an important determining factor in these studies.

The adrenal medulla is part and parcel of a widespread integrated regulatory system that includes the autonomic nervous system and, among other organs, the adrenal cortex and the pituitary. Although the renal excretory response to medullary hormones may be mediated in part by neural pathways, the temporal relationships between stimulus, response, and recovery suggest that humoral mechanisms are operative. Some humoral agents such as antidiuretic hormone and desoxycorticosterone acetate appear to affect renal excretion of water and electrolytes by direct action upon the renal tubules. It is possible that the adrenal medullary hormones behave in this manner or that they alter the production of active agents by other organs. Secondary release of adrenal cortical hormones or adrenocorticotrophic hormone (*25*) might thus account for the increment in tubular reabsorption of sodium, although these substances would be expected to promote—and not depress—potassium excretion (*26*). The marked fall in urine flow on withdrawal of these agents suggests that they may interfere with tubular reabsorption of water, an effect which may be attributable to diminished secretion of antidiuretic hormone (*27*). Perhaps the electrolyte-retaining effect of adrenal medullary hormones is attributable, in part at least, to an altered cellular metabolism. The resultant shifts in electrolyte and water distribution may ultimately influence renal function directly through derangements in the composition of the blood. The relative importance of these factors is under investigation.

Presumably any condition or situation that stimulates release of large amounts of epinephrine and norepinephrine is one in which renal retention of sodium and potassium occurs. However, pheochromocytomata are not reported to have this effect. Hence it is probable that the changes observed in the course of this study occur only as an immediate response and that continued change is prevented by corrective adjustments to restore electrolyte output to normal. It is unlikely, therefore, that epinephrine and norepinephrine play a role in producing the defect in sodium excretion encountered in such conditions as cardiac decompensation. On the other hand, the decrease in electrolyte excretion during anesthesia (*28*) and exercise (*29*), for example, may be ascribed to the action of adrenal medullary hormones upon the tubules as well as to diminished glomerular filtration.[9]

[9] Since this paper was submitted for publication several papers dealing with this subject have appeared. Jacobson, Hammarsten, and Heller (*30*) have confirmed in general the observations reported here. They found that epinephrine (USP) caused a reduction in sodium and

SUMMARY

Epinephrine (USP), *l*-epinephrine, and *l*-norepinephrine have been found to depress the urinary excretion of sodium and potassium in normal human subjects. Since the glomerular filtration rate remained relatively constant despite elevated arterial pressure, intrarenal vasoconstriction, and renal ischemia, the change in electrolyte output must be ascribed to augmented tubular reabsorption. Urine flow usually increased somewhat during the action of the adrenal medullary hormones and fell off sharply on withdrawal, suggesting that these drugs interfere with tubular reabsorption of water.

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potassium excretion. Unfortunately the doses of epinephrine employed produced changes in plasma potassium and glomerular filtration rate that made interpretation impossible. These effects were avoided in the course of the study reported here. Two other papers (31, 32) deal with electrolyte excretion in a small group of subjects given epinephrine (USP) over a period of three days. Sodium output increased whereas potassium output diminished. This phenomenon is not germane to the present discussion but it lends further support to the view expressed above that the acute response is probably of relatively short duration.

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THE INFLUENCE OF CORTISONE UPON THE DIURNAL RHYTHM OF RENAL EXCRETORY FUNCTION

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Vogel in 1854 (1) observed that the flow of urine at night was usually less than that by day. He attributed this nocturnal oliguria chiefly to decreased fluid intake, but Roberts in 1860 (2) and Quincke in 1877 (3) showed that urine flow increased in the morning even when no food or fluid was taken. This matutinal diuresis did not occur if the subject went back to sleep (3). Moreover, although the specific gravity of the night urine was higher than that of the morning urine, the change in density was relatively smaller than that in volume, indicating that excretion of total solids as well as of water was decreased during the night (2, 4–6). Reversal of the normal excretory pattern was described in patients with cardiac or renal disease (5, 7). Similar findings were reported by various authors (8–15). It has also been demonstrated repeatedly that renal excretion rates for chloride (4, 16–32), sodium (4, 23, 24, 29–33), and potassium (4, 23, 24, 29–33) are consistently much lower by night than by day, even when no food or electrolyte is taken or when the dietary intake is equally distributed over each 24-hour period. The output of urea characteristically declines at night (10, 12, 14, 17, 18, 28, 33–35), but Smits (29) and Borst and de Vries (30) relate this decrease in excretion, unlike that of other solutes, to nocturnal diminution of urine flow; they find that the nocturnal excretion rate, "corrected" according to the Van Slyke formula for standard urea clearance, approximates the rate by day. Uric acid excretion also falls during the night (8, 36, 37). Phosphate excretion is ordinarily higher at night than by day (17, 18, 24, 32), but a nocturnal fall has been described in the fasting recumbent subject (38). Although creatinine excretion has been found to decrease slightly at night (19, 36, 37, 39), the change is minute in comparison with the large variations in excretion of water, electrolytes and uric acid. The renal clearances of endogenous creatinine (39, 40) and of inulin (40) normally fall slightly during the nocturnal hours; however, the decline in urine flow at night is almost entirely due to increased tubular reabsorption of water rather than to decreased filtration.

The mechanisms responsible for the normal renal excretory rhythm are obscure. Evidence which suggests that the anterior pituitary is essential for maintenance of some diurnal rhythms in certain species has been reviewed by Kleitman (41). The possible dependence of renal excretory rhythms upon diurnal variations in adrenocortical activity was considered by Borst and de Vries, but they found that nocturnal suppression of sodium and potassium excretion occurred in a patient with Addison's disease (30). Examination of other data concerning electrolyte excretion indicates that the normal diurnal rhythm may be altered in adrenalectomized rats (42) and in untreated patients with Addison's disease (43). On the other hand, of four patients with Addison's disease studied by Slessor (44), three showed a distinct matutinal increase in the rate of chloride excretion, whereas of three patients with panhypopituitarism, two showed no significant increase.

If the diurnal variations in electrolyte excretion are related to fluctuations in adrenocortical activity, they should be modified in patients who receive large doses of cortisone at regular intervals during each 24-hour period. Under these circumstances endogenous production of adrenocortical hormones should be minimal (45–47), and variations in endogenous production would probably be unimportant with continuing absorption from large intramuscular depots, or frequent oral administration. The observations which form the basis of this report indicate that the normal diurnal variations in renal excretion of electrolytes are indeed greatly modified during cortisone ad-

ministration, but that the changes are probably not due to a direct influence of cortisone upon the renal excretory mechanism.

MATERIALS AND METHODS

The subjects were seven male patients, three with rheumatoid arthritis, and one each with asthmatic bronchitis, allergic rhinitis, neurodermatitis, and benign pemphigus. All were free from renal and cardiovascular disease. Each was studied during the administration of cortisone acetate (Cortone, Merck) at six- or 12-hour intervals beginning at 6 a.m. daily. The intramuscular route was employed in all except Z., the patient with pemphigus, who received 50 mg. orally every six hours. Subjects A., C., and D. received 100 mg. every 12 hours, while B., X., and Y. received 125 mg. every six hours. Control observations were made before and after the course of cortisone administration in three patients and after the course of therapy, but not before, in four. One of the subjects was also studied while receiving desoxycorticosterone acetate, 10 mg. intramuscularly every six hours. Three of the patients received each day a diet of unrestricted salt content estimated to contain 130 to 250 meq. of sodium; the others partook of a diet containing approximately 35 meq. of sodium, with or without a supplement of 43 meq. (as 0.45 per cent saline) at 6 a.m., noon, 6 p.m. and midnight. Meals were taken at 7 a.m., noon, and 5 p.m., with free access to water at all times. The patients were ambulatory during the day and remained in bed from 10 p.m. to 6 a.m. Occasional wakefulness at night was controlled by chloral hydrate in doses of 0.6 gm. or secobarbital sodium in doses of 0.1 gm. Except when otherwise noted, pooled specimens of voided urine were collected from 6 a.m. to 10 p.m. and from 10 p.m. to 6 a.m. during each 24-hour period.

In each of two experiments a subject was studied over periods of 48 to 54 hours, during which he remained at strict bed rest and ate identical meals[1] at 6 a.m., noon, 6 p.m. and midnight. Fluid intake was constant (720 or 960 ml.) during each six-hour period and 43 meq. of sodium chloride were ingested at 7 a.m., 1 p.m., 7 p.m., and 1 a.m. Urine collections were made during consecutive

[1] The meals consisted of milk and sandwiches which were made up by taking measured aliquots of single lots of bread, egg salad, cheese and jam. The feedings taken by subject Y. were calculated to contain 28 gm. protein, 36 gm. fat, 72 gm. carbohydrate, 24 meq. sodium and 14 meq. potassium per meal. The meals eaten by subject X. while he was receiving cortisone each contained 30 gm. protein, 40 gm. fat, 72 gm. carbohydrate, 62 meq. sodium and 17 meq. potassium. During his control study each meal contained 23 gm. protein, 25 gm. fat, 85 gm. carbohydrate, 53 meq. sodium and 14 meq. potassium.

TABLE I

Relation of cortisone administration to the diurnal variation of renal excretion in ambulatory patients

Patient and diagnosis	Cortisone dosage (mg./day)	Collection period (inclusive treatment days)	Mean renal excretion rates of									
			Water (ml./min.)		Creatinine (mg./min.)		Na (μ Eq./min.)		Cl (μ Eq./min.)		K (μ Eq./min.)	
			Day*	Night*	Day	Night	Day	Night	Day	Night	Day	Night
A.† Asthmatic bronchitis	200	38th–40th	2.2	2.8	1.20	1.01	158	204	180	220	96	85
	None	+18th–19th	1.1	1.0	1.00	0.90	115	91	135	90	56	22
B.‡ Rheumatoid arthritis	500	10th–13th	1.1	1.7	1.08	1.17	31	67	34	81	56	73
	None	+15th–17th	1.2	0.7	1.07	0.99	40	16	56	21	74	48
C.† Neuro-dermatitis	200	24th–26th	2.5	3.4	1.45	1.17	299	250	347	281	92	97
	None	+11th, 12th, 15th	1.0	0.7	1.33	1.20	215	90	245	85	64	23
D.† Allergic rhinitis	200	15th–17th	5.0	4.7	1.16	1.07	91	177	92	151	62	71
	None	+7th–8th	1.8	4.0	1.47	1.35	265	312	299	283	142	119
X.§ Rheumatoid arthritis	500	13th–16th	2.0	4.1	1.05	0.95	69	195	112	290	66	106
	None	+21st–24th	2.6	1.5	1.01	0.86	144	41	155	53	89	37
Y.§ Rheumatoid arthritis	None	−7th to −4th	2.8	2.0	0.86	0.83	142	73	166	71	66	35
	500	10th	2.2	3.0	0.87	0.84	107	128	150	179	85	98
	500	14th	2.8	4.3	0.99	0.90	115	303	149	351	90	116

* "Day" period, 6 a.m.–10 p.m.; "night" period, 10 p.m.–6 a.m.
† Unrestricted salt intake.
‡ Low salt diet.
§ Low salt diet plus 43 meq. NaCl every six hours.

(+) sign indicates days after discontinuing cortisone.
(−) sign indicates days before instituting cortisone.

TABLE II

Relation of adrenocortical hormone administration to diurnal variation of renal excretion. Subject Z. Benign pemphigus

	Period	Days		Renal excretory rates (mean ± standard error) of										Creat. clear. 6 a.m.-noon	Weight (range)
				Water		Sodium		Chloride		Potassium		Creatinine			
				(*ml./min.*)	P	(*μEq./min.*)	P	(*μEq./min.*)	P	(*μEq./min.*)	P	(*mg./min.*)	P	(*ml./min.*)	(*Kg.*)
I	Low salt*	1–5	D	2.6 ±0.3	<.01	10 ±0.6	<.01	14 ±1.0	<.01	51 ±2.1	<.01	1.38 ±0.04	<.01	111 ±4.8	67.5–68.0
			N	1.3 ±0.1		6 ±0.8		9 ±0.7		30 ±0.9		1.11 ±0.04			
II	Low salt DCA	6–14	D	2.8 ±0.2	<.01	7 ±1.0	<.01	11 ±1.0	<.02	69 ±3.7	<.01	1.46 ±0.02	<.01	111 ±2.2	67.5–68.8
			N	1.5 ±0.1		4 ±0.4		8 ±0.6		48 ±1.0		1.19 ±0.03			
III	Low salt	15–19	D	2.5 ±0.2	<.01	44 ±3.8	<.05	43 ±3.5	<.02	64 ±2.6	<.01	1.39 ±0.03	<.05	119 ±5.7	67.7–68.4
			N	1.5 ±0.1		28 ±4.2		25 ±4.2		39 ±1.8		1.21 ±0.06			
V	Low salt Cortisone†	34–38	D	**2.3 ±0.1**	**>0.1**	**15 ±1.6**	**>.05**	**17 ±1.8**	**>0.3**	**48 ±3.1**	**>0.3**	1.42 ±0.03	<.01	123 ±4.5	66.4–66.8
			N	**2.0 ±0.2**		**21 ±2.1**		**20 ±2.4**		**42 ±4.7**		1.20 ±0.05			
VI	Low salt Cortisone K supplement‡	39–44	D	**2.7 ±0.1**	**>0.1**	**20 ±1.5**	**>0.1**	**21 ±1.7**	**>0.5**	**67 ±12.2**	**>0.9**	1.45 ±0.07	<.05	135 ±6.7	65.6–66.1
			N	**2.4 ±0.1**		**28 ±4.4**		**22 ±5.2**		**67 ±8.7**		1.22 ±0.06			
VII	Cortisone NaCl§ and K supplement	47–59	D	**2.8 ±0.2**	**>0.9**	**116 ±3.2**	**<.01**	**113 ±3.3**	**<.01**	**64 ±2.4**	**<0.1**	1.44 ±0.02	<.01	128 ±4.9	65.8–68.3
			N	**2.8 ±0.1**		**154 ±7.3**		**167 ±8.4**		**72 ±3.4**		1.29 ±0.04			
VIII	Cortisone omitted. NaCl and K supplement	66–72	D	2.8 ±0.2	<.01	124 ±6.0	<.01	132 ±7.3	<.01	71 ±3.5	<.01	1.56 ±0.04	<.05	136 ±8.0	68.2–69.0
			N	1.7 ±0.2		68 ±6.1		68 ±5.5		45 ±2.3		1.39 ±0.04			
IX	Low salt K omitted 79th day‖	76–83	D	2.5 ±0.1	<.01	11 ±1.7	<.01	15 ±1.2	<.01	60 ±1.3	<.01	1.40 ±0.03	<.01	113 ±2.9	68.2–68.8
			N	1.3 ±0.1		5 ±0.5		6 ±0.5		36 ±1.3		1.16 ±0.05			

Data pertaining to Period IV are presented in Figure 4.

P—Probability of observed or greater difference between means of night and day values being found assuming hypothetical difference of zero (as determined by "t" test of group comparison). Bold type indicates that nocturnal excretion rate is *not* significantly *lower* than day rate.

D—Day, 6 a.m.–midnight.

N—Night, midnight–6 a.m.

DCA—Desoxycorticosterone acetate in oil, 10 mg. intramuscularly every 6 hours, days 6 through 14.

* Sodium content approximately 35 meq.

† Cortisone acetate, 50 mg. orally every 6 hours, days 25 through 59.

‡ 500 ml. orange juice at 10 p.m., days 39 through 78.

§ 43 meq. sodium chloride every 6 hours, days 45 through 72.

‖ Excretion rates for K, days 76–78: 57 ± 1.8 (day), 38 ± 4.3 (night), P < .01; days 79–83: 62 ± 1.5 (day), 35 ± 1.1 (night), P < .01.

six-hour periods beginning at 6 a.m. on the first day of the regime. Samples of venous blood were taken at the beginning and end of each six-hour collection period.

Concentrations of sodium and potassium in serum and urine were determined by means of an internal standard flame photometer. Chloride was determined by the modified Volhard-Harvey titration (48). The method of Peters (49) was employed for the determination of creatinine in urine; for serum, the Hare technique (50) was applied. Calculations of endogenous creatinine clearances were based either on the mean of serum concentrations at the beginning and end of each collection period, or on the concentration in a single sample taken at the approximate mid-point of the period.

RESULTS

I. Observations in ambulatory patients

Studies conducted before and after cortisone administration (subject Z.), prior to cortisone therapy (subject Y.), and 11 days or longer after discontinuing cortisone administration (subjects A., B., C., and X.), demonstrated a normal diurnal pattern of renal excretion (Tables I and II). The rate of urine flow was uniformly less by night than by day. The rate of creatinine excretion was slightly depressed at night. Excretion rates of sodium, potassium, and chloride fell markedly at night. The study of subject D., conducted on the seventh and eighth days after cortisone administration had been discontinued, when endogenous adrenocortical activity may well have been suppressed (45–47, 51), will be described below.

All seven subjects displayed striking deviations from normal excretory patterns during cortisone administration. Urine flow at night became approximately equal to the rate by day in subjects D. and Z. and exceeded the day rate in the others. A higher minute output of sodium and chloride by night than by day was observed in all subjects

TABLE III

Diurnal variation of renal excretion in two subjects at bed rest taking identical food and fluids during consecutive six-hour periods

			1st day				2nd day				3rd day
			6 a.m.–12 n.	12 n.–6 p.m.	6 p.m.–12 m.	12 m.–6 a.m.	6 a.m.–12 n.	12 n.–6 p.m.	6 p.m.–12 m.	12 m.–6 a.m.	6 a.m.–9 a.m.
Y.	Control before cortisone	Na *μEq./min.*	106	156	154	77	217	125	172	92	123
		Cl *μEq./min.*	121	148	135	91	239	124	146	95	151
		K *μEq./min.*	37	33	18	12	36	16	17	12	22
		CR *mg./min.*	0.96	0.88	0.85	0.87	0.96	0.78	0.79	0.75	0.82
		Flow *ml./min.*	1.2	1.6	2.2	1.2	1.4	1.8	1.8	1.5	0.9
		C_{CR} *ml./min.*	110	111	114	119	137	115	113	109	121
	Cortisone 11th–13th day	Na *μEq./min.*	24	18	30	37	54	43	85	70	54
		Cl *μEq./min.*	43	34	57	66	93	80	148	116	80
		K *μEq./min.*	45	32	42	43	46	39	46	41	42
		CR *mg./min.*	0.87	0.67	0.65	0.72	0.83	0.74	0.80	0.76	0.84
		Flow *ml./min.*	0.7	0.5	0.9	1.2	0.9	1.1	1.2	1.8	0.7
		C_{CR} *ml./min.*	148	116	112	126	154	145	160	149	165
X.	Control 6 wks. after cortisone	Na *μEq./min.*	167	144	95	94	300	173	117	84	267*
		Cl *μEq./min.*	233	160	89	94	377	192	102	96	292*
		K *μEq./min.*	98	46	32	28	72	34	29	27	55*
		CR *mg./min.*	0.95	0.92	0.93	0.88	0.92	0.93	0.89	0.89	0.77*
		Flow *ml./min.*	2.7	1.9	1.0	1.5	3.5	2.2	1.4	1.3	3.5*
		C_{CR} *ml./min.*	172	167	169	162	170	169	154	162	140*
	Cortisone 23rd–24th day	Na *μEq./min.*	92	159	155	115	156	245	316	163	
		Cl *μEq./min.*	97	158	200	150	184	298	343	201	
		K *μEq./min.*	55	63	73	63	62	78	87	59	
		CR *mg./min.*	0.93	0.92	0.92	0.90	0.97	0.95	0.91	0.80	
		Flow *ml./min.*	1.5	1.8	1.9	1.7	1.8	2.4	2.7	1.7	
		C_{CR} *ml./min.*	158	174	174	177	210	225	217	178	

*6 a.m.–11:35 a.m.

CR—Creatinine; C_{CR}—Endogenous creatinine clearance.

except C. whose nocturnal excretion rates, although markedly increased, remained below the rate by day. Nocturnal rates of potassium excretion were approximately equal to the day rate in A., C., D., and Z. and exceeded the day rate in the others. No significant modification in the pattern of creatinine excretion was noted.

II. Observations during continued recumbency with equally spaced identical meals

Similar alterations in the diurnal excretory rhythm occurred when two of the subjects took identical food and fluid at six-hour intervals while at bed rest. During the control study before cortisone administration, Y. manifested a striking decrease in the excretion of sodium and chloride during the night periods (Table III, Figure 1). Potassium output characteristically increased abruptly during the morning and fell during the afternoon and evening, as well as at night. These variations were unassociated with alterations in creatinine excretion or clearance. While cortisone was being given, a remarkably constant rate of potassium excretion was maintained during nine consecutive periods. The excretion of sodium and chloride exhibited no regular diurnal rhythm, although a distinct rise took place on the morning of the second day and the still higher excretion rates during the period from 6 p.m. to midnight on this day were followed by a decline during the subsequent period from midnight to 6 a.m. Urine flow rose during each night period, while creatinine excretion showed no notable alteration.

During the control study of X., potassium excretion was highest during the morning periods, but while cortisone was being given, a comparatively constant excretion rate was maintained until the second night period (Table III). The unusually small creatinine output recorded for this period suggests that the bladder was incompletely emptied. Creatinine excretion otherwise re-

mained quite constant during both the experimental and control observations. Urine flow declined markedly at night during the control period, but not during cortisone administration. The excretion of sodium and chloride decreased during the periods from 6 p.m. to midnight in the control study but not when cortisone was given. However, lowering of the excretion rates between midnight and 6 a.m. occurred whether or not cortisone was being administered.

III. Serial daily observations before, during, and after cortisone administration

Additional data concerning the temporal relationship of cortisone therapy to the observed changes in renal excretory function were obtained for Y. during eight consecutive days prior to cortisone administration, during the next 14 days while he received cortisone, and during the following 18 days after cortisone was discontinued (Figure 2). When cortisone administration was begun, he excreted little sodium and gained weight. Despite continued administration of the agent, sodium excretion subsequently increased and weight gain ceased.

The changes observed in diurnal excretory rhythm were as follows. The diurnal variation of sodium excretion was altered rapidly; the nocturnal rate approximately equalled or actually exceeded the rate of the antecedent daytime period during and after the 11th day of observation (third day of treatment). This abolition or reversal of the normal diurnal rhythm persisted until the 32nd day (tenth day after cortisone was discontinued) and a consistently normal rhythm was re-established during the 38th through 40th days (16th–18th days after therapy). Changes in chloride excretion are not shown in the figure since they paralleled sodium very closely. The diurnal rhythm of water excretion was eliminated from the tenth through the 38th day. The period during which the diurnal rhythm of sodium output was abnormal (11th–31st days, inclusive) coincided very closely with the time during which body weight was increased, presumably owing to retention of sodium chloride and water. Endogenous creatinine clearance was consistently elevated during most of this period. The marked diurnal variation in potassium excretion, evident during the eight-day pre-treatment control period (except on the third day), was strikingly diminished on the ninth and tenth days (first two days of treatment), and was eliminated or reversed from the 11th through the 24th day (third day of treatment through the second day after treatment). From the 25th through the 33rd day, the pattern varied until a consistently normal rhythm had been restored by the 34th day of observation (12th post-treatment day). Concentrations of serum electrolyte were measured at intervals of approximately one week. During the two weeks of treatment, serum sodium rose from 141 to 147 meq. per liter while potassium fell from 4.7 to 3.2 meq. per liter. During this period, the hematocrit fell from 38 to 34 per cent and hemoglobin from 11.3 to 9.8 gm. per 100 ml. No further change occurred during the first four days after cortisone was discontinued but after two more weeks all values approximated the initial levels.

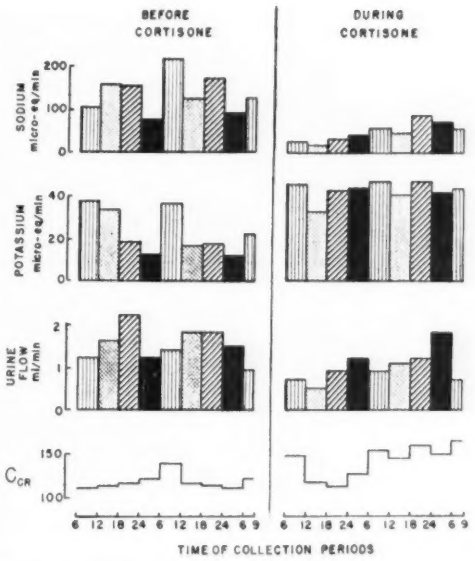

FIG. 1. SUBJECT Y. DIURNAL EXCRETORY PATTERN BEFORE AND DURING CORTISONE ADMINISTRATION WHEN IDENTICAL FOOD AND FLUID WERE TAKEN EVERY SIX HOURS AND RECUMBENT POSTURE MAINTAINED THROUGHOUT

In X., who was studied the day before instituting therapy, during 24 days of treatment and for 24 days after treatment ended, similar changes

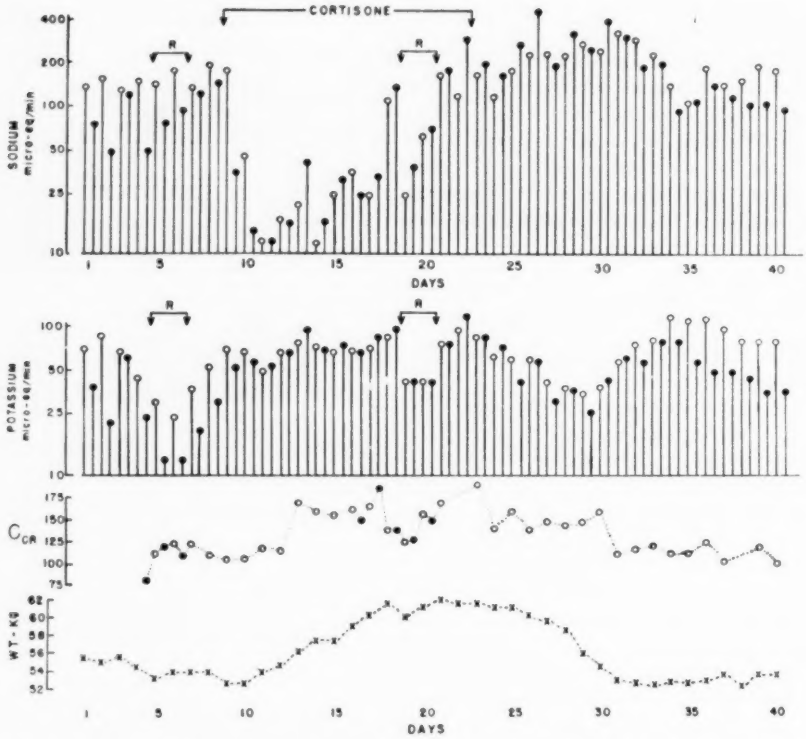

FIG. 2. SUBJECT Y. SERIAL DAILY OBSERVATIONS OF DIURNAL EXCRETORY RHYTHM BEFORE, DURING, AND AFTER CORTISONE ADMINISTRATION

Open circles represent day values. Solid circles, night values. Logarithmic ordinate scale for sodium and potassium excretion rates. During periods marked "R" identical food and fluid were taken every six hours and recumbent posture was maintained.

occurred, but the temporal relationships were somewhat different (Figure 3). Sodium retention was less marked than in Y., and weight gain less rapid. Moreover X., who was treated for ten days longer than Y., continued to excrete large amounts of sodium after the first two weeks and lost 4 kilograms of weight during the last six days of therapy. A normal diurnal rhythm of sodium excretion was maintained during the first ten days of cortisone administration. Reversal occurred abruptly on the 13th day (12th day of treatment) and persisted through the 33rd day (eighth post-treatment day). After three days during which excretory rates by day and night were approximately equal, a small decrease in nocturnal excretion occurred on the 37th day (12th post-treatment day) and a marked nocturnal fall occurred regularly thereafter. Chloride excretion throughout the study was similar to that of sodium and the alterations in the rhythm of water excretion coincided with these also. However, the period of altered excretory rhythm for sodium and chloride did not coincide with increased body weight as in the case of Y. Weight had increased by almost 6 kilograms during the first ten days of treatment without change in the normal excretory rhythm. It is noteworthy that the patient remained at bed rest during this time and did not become ambulatory until the 13th day, after the rhythm had reversed. The return to a normal rhythm after treatment did coincide with the final stabilization of body weight. Al-

though creatinine clearance was not measured before treatment, the available observations suggest that an increase to levels above the normal had occurred by the fourth day of treatment. A gradual decline to normal levels was observed after cortisone was discontinued. The normal rhythm of potassium excretion was eliminated on the fourth day (third day of treatment) and became reversed on the 13th day, remaining so through the 33rd day (eighth day after treatment was discontinued). During the next four days the excretion rates by day and by night were approximately equal; a variable relationship obtained for the eight days following, after which a consistently normal pattern was observed.

The studies on Z. (Table II, Figure 4) were begun while he was taking a diet poor in sodium without the salt supplement received by X. and Y. Following a control period of five days, he was given desoxycorticosterone acetate in oil, 10 mg. intramuscularly every six hours, for nine days. During this time a normal diurnal rhythm persisted (periods I and II, Table II). Increased potassium excretion during DCA administration and increased excretion of sodium and chloride when DCA was discontinued (period III, Table II) were unassociated with any change in the diurnal excretory pattern. Weight changes were minimal during these first three periods as well as after cortisone administration was begun on the 25th day of observation in oral doses of 50 mg. every six hours. The nocturnal decrease in sodium excretion which was regularly apparent before cortisone was given and during the first three days of cortisone therapy was absent or insignificant after the ninth treatment day (Figure

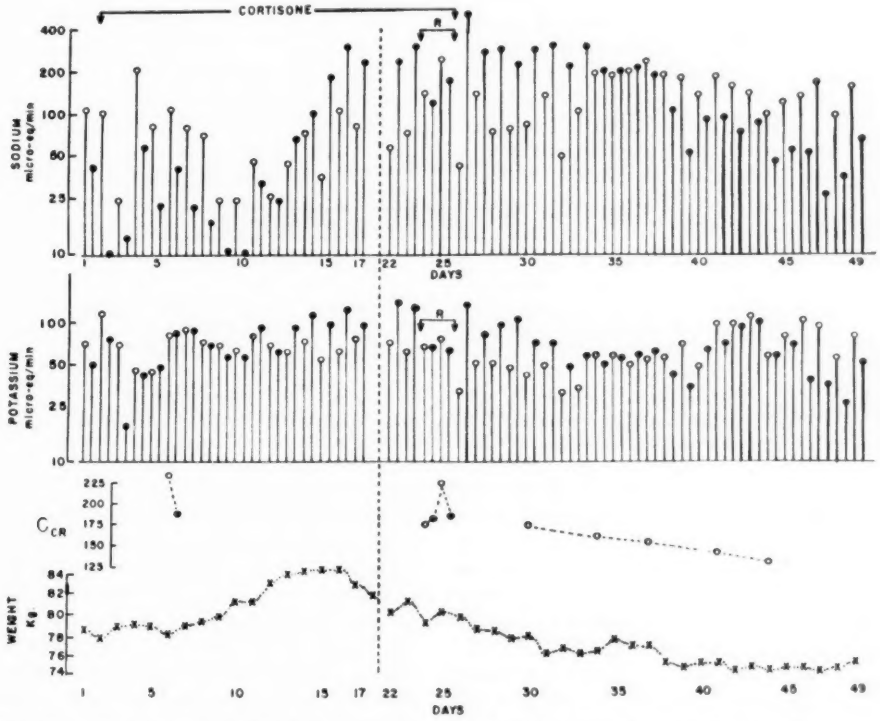

FIG. 3. SUBJECT X. SERIAL DAILY OBSERVATIONS OF DIURNAL EXCRETORY RHYTHM, BEFORE, DURING, AND AFTER CORTISONE ADMINISTRATION

See Figure 2 for legend. The dotted vertical line indicates a period (Days 18 through 21) during which cortisone administration continued but sodium and potassium excretion were not measured.

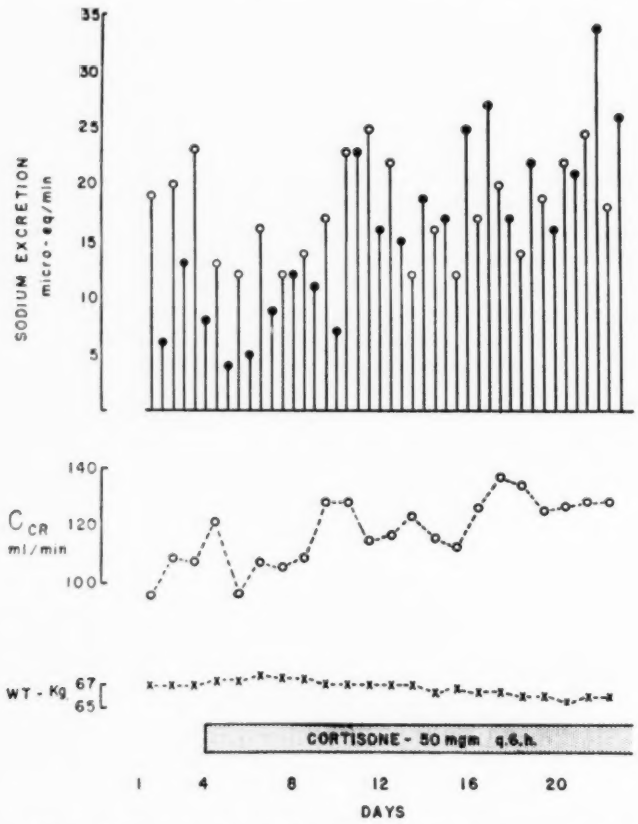

FIG. 4. SUBJECT Z. PERIOD IV. SERIAL DAILY OBSERVATIONS OF DIURNAL EXCRETORY RHYTHM OF SODIUM BEFORE AND DURING CORTISONE ADMINISTRATION

Open circles represent day values. Solid circles, night values. Dietary sodium intake approximately 35 meq. Day 1 corresponds to 22nd day of entire study (Table II).

4). Nocturnal excretion of chloride increased at the same time, while the nocturnal urine flow rose to approximate the day rate 48 hours later. Thereafter, the diurnal rhythm for sodium and chloride was eliminated while salt intake remained low, and reversed when a supplement of sodium chloride was given (periods V–VII, Table II). Return to a normal excretory rhythm for sodium, chloride, and water took place by the 66th day, within a week of discontinuing the hormone (period VIII, Table II). The rhythm of potassium excretion was altered less promptly than in X. and Y. The nocturnal decline in excretory rate was either very small or absent after the 35th day of observation (11th day of therapy). The nocturnal excretory rate regularly equalled or exceeded the day rate after a nocturnal potassium supplement was added to the diet on the 39th day (period VIII, Table II). Despite continued administration of this nocturnal supplement, normal diurnal rhythmicity returned a week after treatment was discontinued and was uninfluenced by change in sodium intake (periods VIII and IX, Table II). Change in endogenous creatinine clearance was far less than in X. or Y. Some increase was observed during the first two weeks

on cortisone (Figure 4), but it is to be noted that the clearance was highest during period VIII, when cortisone had been discontinued and a normal diurnal rhythm had reappeared.

An abnormal excretory pattern after cessation of cortisone administration was observed in D. on his seventh and eighth post-treatment days (Table I). At this time his nocturnal rate of sodium excretion was somewhat higher than the rate by day and the nocturnal excretion of chloride almost equalled that by day. However, potassium excretion was distinctly less by night than by day, although the rate throughout the 48-hour period was remarkably high, presumably because the patient was eating voraciously at the time.

DISCUSSION

The normal diurnal rhythm of renal excretory function can be demonstrated under a variety of experimental conditions and is not consistently altered by reversing or otherwise modifying the pattern of sleep and activity by day and by night (18, 19, 25, 30, 52–54). The observation that when cortisone was administered the normal rhythmic excretory pattern was virtually abolished or actually reversed in each of the seven subjects indicates that this agent exerts an important action upon the mechanism responsible for the normal rhythm.

That the posture of the subject continued to exert an important influence during cortisone administration is demonstrated by the fact that the two subjects who remained recumbent throughout a 48-hour period during therapy failed to exhibit a reversal of the excretory rhythms for sodium and chloride, although the normal nocturnal suppression of excretion was decreased or eliminated. However, when these two subjects were ambulatory by day actual reversal of rhythm occurred, as it also did in four of the other five subjects who remained recumbent only during the night. In the normal individual, not receiving cortisone, assumption of the recumbent posture during the day characteristically evokes a diuresis of water, sodium, and chloride (4, 5, 15, 21, 30, 31, 55–69). A similar diuresis would be expected to occur during nocturnal recumbency if the factors which maintain the normal diurnal rhythm were not operative. Modification of these factors by cortisone administration appears to permit recumbency to evoke at night the renal response that it normally induces only during the day.

There was no clear influence of posture on the change in the diurnal rhythm of potassium excretion which was eliminated or reversed in all seven subjects. Other observations concerning the renal excretion of potassium (30, 69, 70) suggest that the influence of posture is unimportant.

Although an increase in the rate of endogenous creatinine clearance occurred during therapy (71), the absence of significant alterations in the normal small diurnal variation in its clearance rate argues against the possibility that altered patterns of water and electrolyte excretion were related to changes in the diurnal rhythm of glomerular filtration. Moreover the elevation of clearance could hardly explain the disturbance of diurnal rhythm. In subject Y. the pattern of potassium excretion was altered before creatinine clearance rose. In X. creatinine clearance was not measured before cortisone was given. The clearance determined on the fifth day of cortisone administration was strikingly elevated (230 ml./min.), but reversal of the diurnal rhythm for sodium and chloride did not occur until the 12th day of treatment. In Z. changes in the magnitude of clearance were small and apparently unrelated to change in diurnal rhythm. The validity of endogenous creatinine clearance as a measure of glomerular filtration rate in subjects receiving ACTH or cortisone is open to question (72), but the dissociation of the changes observed in the magnitude of creatinine clearance on the one hand, and the pattern of electrolyte excretion on the other, suggests that they are not directly interdependent.

Alterations in the patterns of excretion of sodium and of chloride were apparent both when the diet was poor in sodium and when the dietary salt intake was liberal. Conceivably, reversal of the excretory rhythm for sodium and chloride by cortisone might be related to concomitant or antecedent cumulative retention of these ions, since a similar reversal is observed when they are retained in patients with congestive heart failure (30, 33, 73, 74) or cirrhosis of the liver (29, 30, 33). In the studies of Y. the period during which reversal of diurnal rhythm was apparent coin-

cided quite closely with the period of sodium retention. The studies of Z., however, demonstrate that such an association is not a necessary one, since reversal of rhythm took place while he received a salt-poor diet without gain in weight or decrease in sodium excretion.

The temporal relationships of the alterations in excretory rhythm of sodium and chloride to the administration of cortisone, as defined in the studies of X., Y. and Z., afford strong evidence that the changes cannot be attributed to an immediate direct influence of cortisone upon renal excretory function. Subjects X. and Z. continued to manifest a normal diurnal pattern for more than a week after they had been receiving cortisone in daily doses of 500 mg. Even on the 23rd and 24th days of treatment, X. exhibited a nocturnal fall in excretion rates of sodium and chloride during constant recumbency. In another subject (C., Table I), the nocturnal excretion of sodium and chloride remained below the rates by day after more than three weeks of cortisone administration. Persistence of abnormalities in the rhythm of sodium and chloride excretion for seven to 16 days after cortisone had been discontinued provides additional evidence that alteration in rhythm is not dependent upon the presence of large amounts of cortisone in the organism; indeed, during this post-treatment period a deficient supply of adrenocortical hormones is often evident (45–47, 51).

The temporal relationship of change in the pattern of potassium excretion to administration of cortisone was somewhat more suggestive of a direct influence upon the renal excretory rhythm in subject Y. whose nocturnal excretion rates approached the day rates on the first two days of treatment and thereafter equalled or exceeded the rates by day. In X. the normal rhythm was also eliminated on the third day of treatment. However, Z. maintained a normal rhythm during the first ten days of cortisone administration and all three subjects manifested an abnormal excretory rhythm for at least a week after cortisone had been discontinued.

These temporal dissociations between cortisone administration and alteration in diurnal rhythm do not support the hypothesis that the normally occurring rhythm is determined by a diurnal rhythm of endogenous adrenocortical secretory function.[2] Other considerations also argue against this hypothesis. First, increased endogenous production of adrenocortical hormone due to increased liberation of ACTH during the morning might be expected to induce a decrease in excretion of sodium and chloride rather than the increase normally found. Stanbury and Thomson were able to produce a nocturnal increase of potassium excretion by giving a single dose of ACTH at night, but the excretory rhythm for sodium was unchanged (32). Second, although the adrenalectomized rat maintained on tap water fails to manifest the nocturnal rise of sodium excretion characteristic of intact members of this species, when saline is given, the excretory pattern of sodium is normal (42). Third, the data of Levy, Power, and Kepler (43) indicate that in untreated patients with Addison's disease the excretion of chloride and sodium increases slightly in the morning and that this matutinal increase attains normal magnitude during treatment with adrenal cortical extract or desoxycorticosterone acetate. The observations of Borst and de Vries (30) and of Slessor (44) afford additional evidence that patients with Addison's disease excrete less sodium and chloride at night than by day. Finally, a patient with Addison's disease has been studied while maintained on desoxycorticosterone acetate (1.25 mg. intramuscularly at 6 a.m. and 6 p.m.) and cortisone (6.25 mg. orally at 6 a.m. and 6 p.m.) (85). When he remained at bed rest and took identical food and fluid every six hours day and night, the

[2] The existence of such an adrenocortical rhythm in man is suggested by observations that the renal excretion of 17-ketosteroids (75–79), neutral reducing lipids (78), and uric acid (8, 36, 37) is less at night than during the day, as well as by the fact that the numbers of circulating lymphocytes (80) and eosinophils (81–83) increase at night. It is recognized that the diurnal excretory rhythm of 17-ketosteroids and neutral reducing lipid could occur even with a constant rate of endogenous secretion of adrenocortical hormones if there were appropriate variations either in the rate at which conversion of these precursors to their excretion products took place or in the renal clearance of the excretion products (79). However, preliminary observations indicate that when large amounts of a precursor (testosterone propionate) are supplied exogenously at 12-hour intervals to normal subjects, the additional excretion of 17-ketosteroids is not significantly greater during the day than at night (84).

diurnal excretory pattern for water, sodium, chloride and potassium was essentially normal.

The above evidence permits the conclusion that the diurnal rhythm of excretion of sodium and chloride is not directly dependent upon endogenous adrenal activity, even though adrenocortical hormones may exert profound indirect influences. Nor is it probable that the normal excretory rhythm for potassium is determined by a diurnal adrenocortical rhythm. The temporal dissociation between cortisone administration and change in excretory rhythm, although less striking than in the case of sodium, is hardly consistent with the belief that the large diurnal changes in excretion of potassium which normally take place over a very few hours could be induced by change in endogenous production of cortisone-like steroid. Moreover, although in Z. the administration of desoxycorticosterone acetate at six-hour intervals evoked a large increase in potassium excretion, the nocturnal rates remained well below those of the day. The patient with Addison's disease studied by us exhibited a normal pattern of potassium excretion when he remained recumbent throughout a 72-hour period. Borst's and de Vries' patient exhibited a similar normal rhythm, although the magnitude of variation was less than that of a single control subject maintained under similar conditions (30). Matutinal increase in potassium excretion was absent in the untreated patients with Addison's disease reported by Levy, Power, and Kepler, but in treated patients an increase took place, although this was less than in normal subjects (43).

It would appear, therefore, that the diurnal rhythm of renal excretory function is altered by cortisone in some indirect manner. Since hypokalemic alkalosis may occur during its administration, serial determination of serum bicarbonate in the patients studied would have been of interest, particularly in view of Stanbury's and Thomson's suggestion that the excretory rhythm may be linked with cyclic changes in acid-base equilibrium (32). Alterations in the total daily excretion of water and solutes such as urea and phosphate related to increased food intake and protein catabolism during cortisone administration might induce changes in the diurnal excretory pattern. Further observations are required, but the persistence of abnormalities in the diurnal rhythm after cortisone withdrawal would make it difficult to relate the changes to such metabolic effects. The possibility that a secondary effect on the adenohypophysis is involved might be considered, since there is some evidence that the anterior pituitary plays a role in other diurnal rhythms (41), and that the excretory rhythm for chloride may be abnormal in patients with panhypopituitarism (44). Mills has suggested that rhythmic activity of the hypothalamus may be responsible for diurnal variation in urine flow (53). Since cortisone may have pronounced cerebral effects (45, 46), including disturbance of normal sleep patterns, and since the central nervous system appears to have important influences on the excretion of electrolyte as well as of water (86–92), it is possible that its neurological action may be involved in producing the changes we have observed.

SUMMARY AND CONCLUSIONS

1. The influence of cortisone administered in divided daily doses of 200 or 500 mg. upon the diurnal variation in renal excretion of water, sodium, potassium, chloride, and creatinine has been studied in seven patients free from renal or cardiovascular disease. One of these patients was also observed during the administration of desoxycorticosterone acetate in divided daily doses of 40 mg.

2. The diurnal excretory rhythm was not altered during administration of desoxycorticosterone.

3. During cortisone therapy when the subjects were ambulatory, the normal diurnal rhythm of renal water excretion was reversed in five of the seven patients and virtually eliminated in the others. The excretory rhythm of sodium and chloride was reversed in six patients. The normal rhythm of potassium excretion was eliminated in all seven and reversed in three of these. No significant change occurred in the pattern of creatinine excretion.

4. In two subjects at bed rest who ate identical meals every six hours, the diurnal excretory rhythm for potassium was eliminated in both, but one continued to manifest nocturnal suppression of sodium and chloride excretion.

5. The temporal sequence of the changes was studied in three of the subjects before, during,

and after the course of cortisone administration. The observations seem most consistent with the belief that the changes in excretory rhythm are not the result of a direct action of cortisone upon the renal excretory mechanism.

6. The normal diurnal rhythm of renal excretion does not appear to depend upon rhythmic changes in endogenous adrenocortical secretion, although the demonstration that striking changes occur when large amounts of exogenous adrenal steroid are given suggests that endogenous adrenal hormone may also exert important modifying influences.

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STUDIES ON A PROTEOLYTIC ENZYME IN HUMAN PLASMA. VII. A FATAL HEMORRHAGIC STATE ASSOCIATED WITH EXCESSIVE PLASMA PROTEOLYTIC ACTIVITY IN A PATIENT UNDERGOING SURGERY FOR CARCINOMA OF THE HEAD OF THE PANCREAS[1]

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Human plasma contains a proteolytic enzyme system which can digest fibrinogen and fibrin. This enzyme system appears to be in an inactive form in the circulating plasma, but its presence can be demonstrated in a number of ways in shed blood. This *in vitro* proteolytic activity seems to be influenced by the physiologic state of the subject from whom the blood is obtained. Abnormally rapid fibrinolysis has been observed in blood drawn from patients with a wide variety of diseases (1–3). Rapid fibrinolysis has also been seen in the blood of patients undergoing electric shock therapy (4), and in patients with shock as the result of burns or hemorrhage (5).

The possibility that a hemorrhagic state may result from the destruction of prothrombin or fibrinogen by the plasma proteolytic enzyme has been postulated repeatedly (5–7). A number of patients have been described in whom a hemorrhagic state was associated with excessive plasma fibrinolytic activity (5, 8, 9). These patients were thought to have little or no fibrinogen in their circulating plasma.

Additional evidence that hemorrhage may be associated with activity of the proteolytic enzyme of plasma has been provided by the study of a patient with carcinoma of the head of the pancreas. During the second stage of a pancreatectomy, uncontrollable hemorrhage occurred from multiple sites. The patient's blood, obtained two and a half hours before death, contained appreciable amounts of prothrombin and fibrinogen. However, the patient's whole blood failed to clot in glass tubes at 37° C. A circulating anticoagulant was not demonstrable, but her plasma appeared to have extreme fibrinogenolytic and fibrinolytic properties. It was concluded that the patient's blood failed to clot because the fibrinogen it contained was destroyed before any detectable quantity of fibrin formed.

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CASE REPORT

N. J. (Mt. S. No. 7640), a 42 year old colored woman, was hospitalized on July 27, 1951 because of right upper abdominal pain and soreness for the preceding week. She had always bruised easily, had twice bled profusely after dental extractions, and had had one severe epistaxis. The patient had rectal bleeding, first noted in 1948, associated with external and internal hemorrhoids. A barium enema and sigmoidoscopy revealed no abnormality.

In April, 1950, her bleeding time was one minute, clotting time was three minutes (capillary tube method), prothrombin time was 14.8 seconds, the platelet count was 146,300 and clot retraction was observed in 45 minutes. These values were normal for the techniques then in use.

The remainder of the patient's past history was not unusual. A thyroid adenoma was observed in August, 1950; the basal metabolic rate was plus two. There was no history of menorrhagia or metrorrhagia. Her 10 pregnancies were without hemorrhagic complications. There was no family history of hemorrhagic disease.

For the five weeks before her hospitalization, the patient had intermittent right upper quadrant pain and tenderness, excessive eructation, and anorexia. She was treated during this period with a low fat diet and lost about 13 pounds. During the week before admission, she had nausea, "heart burn" and transient diarrhea. Four days before admission her urine was dark and her stools white. Her last menses began 10 days before hospitalization and lasted seven days, an excessively long time for the patient.

At the time of admission, the patient did not appear to be ill. The sclerae were icteric and the edge of the liver was palpated about 5 cm. below the costal margin; two observers thought the tip of the spleen was palpable. The urine was brown and contained bile pigments; a two hour specimen had from 0.2 to 0.7 Ehrlich units of urobilinogen. The red blood cell count was 4.27 million, the hemoglobin was 12.5 grams (Sahli), and the hematocrit,

41 per cent. The white blood cell count was 10,350; the differential count was unremarkable and platelets were plentiful in the smear. The stools were clay colored and tests for stercobilin and stercobilinogen were negative. The serologic test for syphilis was negative. The concentration of bilirubin in the serum was 9.0 mg., albumin, 4.9 grams, globulin, 3.1 grams, non-protein nitrogen, 25 mg., total cholesterol, 187 mg., esterified cholesterol, 78 mg., and sugar, 114 mg. per 100 ml. The cephalin cholesterol flocculation test was four plus and the thymol turbidity was 1.7 Maclagan units. The serum alkaline phosphatase activity was 16.9 Bodansky units and gradually increased during the next two weeks to 30.6 units. The prothrombin time was 17.9 seconds; the control time was 15.7 seconds. On August 17, the plasma fibrinogen concentration was 555 mg. per 100 ml., an abnormally high value. X-rays of the chest, stomach and upper intestine showed no abnormalities. The gall bladder could not be visualized on two occasions by oral cholecystography.

On August 20, 1951, an exploratory laparotomy revealed chronic pancreatitis and an adenocarcinoma of the pancreas invading the first portion of the duodenum. The distal one-fourth of the stomach was removed, a gastroenterostomy and cholecystojejunostomy were performed, and a T-tube was placed in the common duct. During the operation, almost continuous oozing of blood was noted from the exposed serosal surfaces. Nine days post-operatively, cholangiograms revealed a fusiform narrowing of the common duct distal to the T-tube.

On September 13, the clotting time was 21 minutes, a normal value, and the prothrombin time was 20.1 seconds, compared with a normal of 16.8 seconds.

A second operation was performed September 14. After premedication with seconal, demerol, and scopolamine, anesthesia was induced with pentothal and maintained with cyclopropane. The scar of the previous incision was excised, and the incision was extended to a row of interrupted wire sutures inserted at the previous operation. These wires were removed without excessive bleeding. A transfusion of citrated bank blood was begun at this time, 15 minutes after the start of the operation. The abdominal cavity was then entered preparatory to a pancreatectomy. Forty-five minutes after the beginning of the operation, while the surgeon was freeing adherent loops of intestine to reach the pancreas, the patient's blood pressure suddenly fell from 130/80 to 80/50 mm. of mercury. The pulse rate was 95 per minute and the skin was warm and dry. Blood began to ooze from the omental and serosal surfaces, but a discrete bleeding point could not be found. The hemorrhage grew progressively more profuse, and was uncontrollable. The blood pressure gradually fell, and the patient expired five hours after the operation had begun. At the time of death, approximately 5 liters of unclotted blood had collected in the peritoneal cavity. The patient had hematomas at the sites of venous and arterial punctures, and blood flowed from a Levine tube inserted into the stomach. Among the unsuccessful measures used to control the patient's hemorrhages were the transfusion of 4,000 ml. of citrated bank blood, and the intravenous injection of 10 ml. of a 10 per cent solution of calcium gluconate and 5 ml. of a one per cent solution of protamine sulfate.

The patient's blood was Group O Rh_1 Rh_2. The blood of each of the eight blood donors was also Group O, Rh positive. No incompatibility was demonstrable between the patient's serum and any of the donors' cells, or *vice versa*. The cross-matchings were rechecked *post mortem* by both slide and tube methods, using both saline and 30 per cent bovine albumin as diluents. The donors were retyped, and their bloods were again compatible with the patient's. The patient's urine, obtained *post mortem*, was negative for hemoglobin when tested with orthotolidene and benzidene. Her plasma, about 120 minutes after the initial fall of blood pressure, contained 18 mg. of hemoglobin per 100 ml.

The initial donor's blood contained agglutinins in a titer of 1:16 against trypsin-treated normal Group O, Rh positive and Rh negative red blood cells, and the patient's trypsin-treated red cells. One week later, a fresh specimen of this donor's blood contained agglutinins against trypsin-treated red cells in a titer of 1:4. The direct and indirect Coombs tests of the patient's blood and each of the donors' bloods were negative.

At the operation, the diagnosis of adenocarcinoma of the pancreas was confirmed by biopsy. The tumor cells had invaded both lymphatic and venous channels. A specimen of liver was smooth, glistening and yellowish-green. Histologically, there was early biliary cirrhosis. Permission for autospy was not obtained.

METHODS

Three hours after the induction of anesthesia, two hours after bleeding was first noted, blood was drawn from the right jugular vein through a No. 18 gauge needle, using silicone-treated equipment. At this time, the patient had already received approximately 3,000 ml. of citrated blood. A portion of the blood drawn was used to measure the whole blood clotting time. Another portion was transferred to silicone-lined lusteroid tubes and the cells were removed by high speed centrifugation at low temperatures (10, 11). This platelet-deficient native plasma contained one platelet per mm^3. Still another portion of blood was decalcified by mixing one part of 0.1 M. sodium oxalate with nine parts of whole blood, centrifuged, and the supernatant plasma used in all tests requiring oxalated plasma except the determination of fibrinogen. A fourth portion was mixed with dry oxalate salts in a proportion of 5 ml. of whole blood to 6 mg. of ammonium oxalate and 4 mg. of potassium oxalate. This oxalated plasma was used to measure plasma fibrinogen.

Determination of clotting time. One ml. of blood was transferred immediately to each of three dry pyrex tubes (internal diameter, 11 mm.) which were stoppered and incubated at 37° C. Starting five minutes after the blood was drawn, the first tube was observed for clotting by tilting it about once a minute. Sixty, 120, and 180 min-

utes after the blood was drawn, the second and third tubes were also tilted. Thereafter, the third tube was examined frequently for 24 hours. The clotting time of platelet-deficient plasma was determined in the same way in duplicate. After the contents of the first tube had clotted, the second was tilted about once a minute for an additional 90 minutes.

Determination of prothrombin time. The prothrombin time was determined in duplicate at 37° C. by mixing 0.1 ml. of oxalated plasma and 0.2 ml. of equal parts of thromboplastin (Chilcott) and 0.025 M. calcium chloride, in pyrex tubes (internal diameter, 8 mm.).

Determination of fibrinogen content of plasma. The fibrinogen content of plasma was determined by a previously described method (12).

Preparation of fibrinogen solution. Desiccated bovine fibrinogen [2] was dissolved in barbital buffer (0.125 M. sodium chloride and 0.025 M. barbital at pH 7.5), and the undissolved small residue was removed by filtration through No. 1 Whatman filter paper. In each experiment, then, the concentration of fibrinogen was something less than the recorded concentration.

Determination of fibrinolytic activity of plasma. Oxalated plasma was diluted serially with barbital buffer. Three-tenths ml. of diluted plasma was mixed with 0.3 ml. of a solution of bovine fibrinogen (330 mg. per 100 ml.) in pyrex tubes (internal diameter, 8 mm.). This mixture was then clotted with 0.1 ml. of a solution of bovine thrombin [3] (10 N.I.H. units per ml.). The tubes were stoppered, incubated at 37° C., and observed for lysis.

Determination of inhibitory activity of plasma against plasma proteolytic enzyme. The inhibitory activity of plasma against the plasma proteolytic enzyme was tested against purified bovine plasma proteolytic enzyme.[4] The enzyme was dissolved in buffer and standardized in the following manner: The enzyme solution was serially diluted with buffer, 0.3 ml. of each dilution was pipetted into pyrex tubes (internal diameter, 8 mm.) and mixed with 0.3 ml. of buffer and 0.1 ml. of a solution of thrombin (10 N.I.H. units per ml.). The tubes were placed in an ice bath, and 0.3 ml. of fibrinogen solution (330 mg. per 100 ml.) was added. The mixtures were then transferred to a water bath at 37° C., and the clots which formed were observed for lysis. The concentration of bovine plasma proteolytic enzyme which completely lysed this standard fibrin clot in 10 minutes was 2.5 units per ml.

The patient's oxalated plasma was serially diluted with buffer, and 0.3 ml. of each dilution was mixed with 0.3 ml. of a solution of bovine enzyme (2.5 units per ml.) and incubated at 37° C. for 10 minutes. The mixtures were iced and 0.3 ml. of fibrinogen and 0.1 ml. of thrombin were added. The tubes were then incubated at 37° C., and the clots which formed were observed for lysis.

Determination of anticoagulant activity of plasma. The anticoagulant activity of plasma was tested by measuring its clot-retarding effect on normal blood (13).

Determination of thrombin-inhibitory activity of plasma. The clotting time of equal parts of the patient's plasma, a solution of bovine fibrinogen (250 mg. per 100 ml.), and a solution of bovine thrombin, serially diluted, was measured at 37° C. in pyrex tubes (internal diameter, 8 mm.). The clotting time of this mixture was also determined, replacing fibrinogen with prothrombin-deficient plasma, prepared by incubation with one-fifth its volume of powdered barium sulfate at 37° C. for 10 minutes.

RESULTS

Venous blood, drawn two hours after the patient began to bleed, failed to clot in glass tubes within 24 hours. Platelet-deficient native plasma was prepared from blood drawn at the same time and its clotting time was determined in duplicate. Strands of fibrin appeared within 28 minutes in the first tube, and clotting appeared to be complete 10 minutes later. This clot did not seem as solid as that of normal plasma, but it was not displaced when the tube was inverted. At this time, observation of the second tube was begun. No clot was seen in this second tube during the next 90 minutes. Eleven minutes after the plasma in the first tube had clotted, the clot lysed completely. These observations suggested that the patient's plasma contained those elements necessary for clotting to take place within a normal time, but it possessed excessive fibrinolytic activity.

The fibrinolytic activity of plasma is due to the presence of a proteolytic enzyme which can also digest prothrombin (14) and fibrinogen. The possibility was studied that whole blood failed to clot because of the activity of this enzyme. Three hours and 15 minutes after the blood was drawn, 0.1 and 0.5 ml. of a solution of bovine thrombin (1,000 N.I.H. units per ml.) were added, respectively, to the unclotted blood in the first and second tubes used to determine the clotting time. No clots were seen in either tube during the next four days. Similarly, 108 minutes after the start of the test for the clotting time of platelet-deficient plasma, the addition of 100 units of thrombin did not result in clotting in the second, unclotted tube.

There are at least two interpretations of these experiments. Either the blood failed to clot due

[2] Bovine fibrinogen was obtained through the courtesy of Dr. Walter Seegers.

[3] Bovine thrombin was obtained through the courtesy of Parke, Davis & Company, Detroit.

[4] Bovine fibrinolysin (plasma proteolytic enzyme) obtained through the courtesy of Parke, Davis & Company, Detroit.

TABLE I

The effect of thrombin on the clotting time of a mixture of oxalated plasma and fibrinogen

Clotting time of a mixture of 0.1 ml. of oxalated plasma, 0.1 ml. of a solution bovine fibrinogen (containing a maximum of 250 mg. per 100 ml.), and 0.1 ml. of a solution of bovine thrombin, serially diluted with buffer.

Initial Concentration of Thrombin	Clotting Time	
	Patient's Plasma	Control Plasma
units/ml.	sec.	sec.
10	14	9
5	18	16
2.5	28	26

to a deficiency in some element needed for clotting, or to the presence of something interfering with the action of thrombin.

Since one of the samples of platelet-deficient plasma clotted, it seemed unlikely that excessive thrombin-inhibitory activity was present. When small amounts of thrombin were added to oxalated plasma, the clotting time of the patient's plasma was longer than that of a control plasma studied at the same time. The clots which formed when thrombin was added to the patient's plasma dissolved within five minutes, while those formed from normal plasma did not dissolve within the next 24 hours. In these experiments, the prolonged clotting time of the patient's plasma was more probably related to the small amount of fibrinogen present than to a thrombin inhibitor. The clotting time of a mixture of thrombin and fibrinogen decreases as the concentration of fibrinogen increases until a minimum time is reached. To eliminate the influence of the fibrinogen concentration on the clotting time, the patient's plasma was mixed with an equal portion of a solution of bovine fibrinogen and clotted with thrombin at 37° C. Sufficient fibrinogen was added to make the clotting time independent of its concentration. With this technique, the thrombin-inhibitory activity of the patient's plasma was normal (Table I). Normal thrombin-inhibitory activity was also observed when the patient's

TABLE II

Fibrinolytic activity of oxalated plasma

Fibrinolysis (+) observed in a mixture of 0.3 ml. of oxalated plasma, serially diluted with buffer, 0.3 ml. of bovine fibrinogen solution (maximum concentration, 330 mg. per 100 ml.) and 0.1 ml. of a bovine thrombin solution containing 10 u. per ml. The mixture was incubated at 37°C. for 16 hours.

SOURCE OF PLASMA	INITIAL CONCENTRATION OF PLASMA					
	1/10	1/20	1/40	1/80	1/160	0
PATIENT	+	+	+	+	±	0
NORMAL CONTROL	0	0	0	0	0	0

plasma was mixed with normal human plasma from which the prothrombin had been removed.

The foregoing observations showed that the patient's plasma contained fibrinogen which could be clotted by thrombin, and, in one of two samples of native plasma, clotted spontaneously. Paradoxically, whole blood failed to clot, and after incubation at 37° C. for 205 minutes, did not clot when thrombin was added. A reasonable inference was that the fibrinogen in the patient's whole blood was digested by the plasma proteolytic enzyme before visible clotting took place. That this was likely, was demonstrated in the following manner. The concentration of fibrinogen was measured in a sample of oxalated plasma which had been separated from the blood cells by centrifugation at room temperature. The fibrinogen was clotted with thrombin, and the fibrin washed three times with sodium chloride solution. Ninety minutes elapsed from the time the blood was drawn until the washed fibrin was digested with sodium hydroxide. The concentration of fibrinogen in this sample was 39 mg. per 100 ml.

Two hours and ten minutes after the blood was drawn, when it became evident that the patient's plasma was excessively fibrinolytic, the fibrinogen determination was repeated using native plasma which had been kept at 0° C. or less throughout this period. The fibrinogen in this plasma was clotted with thrombin; the fibrin was kept at room temperature for about 20 minutes while it was washed. The concentration of fibrinogen in this second specimen was 105 mg. per 100 ml. Thus, the amount of fibrinogen which seemed to be present in the patient's plasma decreased as it stood at room temperature.

The ability of the patient's plasma to lyse fibrin clots was measured directly. Mixtures of bovine fibrinogen and varying concentrations of plasma were clotted with bovine thrombin, and the clots were incubated at 37° C. for 16 hours. The patient's plasma was fibrinolytic even when it was diluted almost 400 times (Table II). On the other hand, it was as effective as normal plasma in inhibiting the fibrinolytic activity of bovine plasma proteolytic enzyme (Table III), although this inhibition was observed only when the plasma was diluted beyond the point where its fibrinolytic activity was demonstrable.

The patient's blood was studied for the presence of other defects in the clotting mechanism.

TABLE III

Protease inhibitory activity of oxalated plasma

Clot lysis time at 37°C. of a mixture of 0.3 ml bovine plasma proteolytic enzyme solution, 0.3 ml. oxalated plasma, serially diluted with buffer, 0.1 ml. bovine thrombin solution (10 units per ml.), and 0.3 ml. bovine fibrinogen solution (maximum concentration, 330 mg. per 100 ml.). The enzyme and plasma were incubated at 37°C. for 10 minutes before the addition of thrombin and fibrinogen.

Initial Concentration of Plasma	Concentration of Plasma Proteolytic Enzyme	CLOT LYSIS TIME		
		Patient's plasma	Control Plasma	Buffer*
	u/ml.	min.	min.	min.
1/100	2.5	30	30	--
1/200	2.5	16	16	--
1/400	2.5	14	13	--
0	2.5	--	--	9
0	1.25	--	--	30

*Substituted for oxalated plasma.

TABLE IV

Effect of oxalated plasma on the clotting time of recalcified hemophilic plasma

MIXTURE	1	2	3
Normal Oxalated Plasma, ml.	0.	0.1	0
Patient's Oxalated Plasma, ml.	0	0	0.1
Hemophilic Oxalated Plasma*, ml.	0.2	0.1	0.1
$CaCl_2$ 0.025 M	0.2	0.2	0.2
Clotting Time (Min.)	150	4	6

*The hemophilic plasma was 17 days old.

The platelet count was 62,000/mm³. The prothrombin time was 28.6 seconds compared with a control of 16.8 seconds. This prolongation of the prothrombin time might have been due to a deficiency in prothrombin or in some other clotting element. The clotting time of a mixture of equal portions of the patient's oxalated plasma, normal prothrombin-deficient plasma, thromboplastin and calcium chloride was 30.3 seconds, compared with a control of 24.6 seconds. Presumably, therefore, hypoprothrombinemia was present in the patient's plasma when the test was performed.

The plasma proteolytic enzyme has not been reported to digest the substance lacking in hemophilic plasma. The recalcified clotting time of stored hemophilic plasma was shortened almost as much by the patient's plasma as by normal plasma (Table IV).

None of the experiments thus far described suggested that the patient's plasma contained a circulating anticoagulant. None the less, the patient's platelet-deficient plasma prolonged the clotting time of normal whole blood (Table V). This effect was probably due to plasma's excessive proteolytic activity rather than to an anticoagulant. Twenty-four hours later, the patient's platelet-deficient plasma no longer prolonged the clotting time of normal whole blood.

DISCUSSION

A hemorrhagic state due to the destruction of prothrombin or fibrinogen by the proteolytic enzyme of plasma is a very rare phenomenon. Tagnon, Levenson, Davidson, and Taylor (5) described a woman who died after a severe *post partum* hemorrhage despite transfusion of blood and hysterectomy. A mixture of fibrinogen and the patient's plasma clotted, but the clot dissolved within one hour. The case of another woman who had a severe uterine hemorrhage associated with *abruptio placentae* was reported by Weiner,

TABLE V

Effect of patient's native plasma on the clotting time of normal blood

Clotting time at 37°C. of 1 ml. portions of normal blood to which was added buffer or the patient's plasma in the amounts indicated. Triplicate determinations.				
0.1 ml. Buffer	0.5 ml. Buffer	0.1 ml. Pt's Plasma	0.2 ml. Pt's Plasma	0.5 ml. Pt's Plasma
Min.	Min.	Min.	Min.	Min.
9	13	21	9	28
14	15	24	13	31
17	18	24	18	39

Reid, and Roby (8). Six hours after the onset of hemorrhage, when the patient had received 1,500 ml. of blood, a specimen of blood seemed to gel in six minutes but 15 minutes later was completely liquid and no fibrinogen could then be detected in the specimen. A lysin which digested a fibrin clot was demonstrable. No other bleeding phenomena were observed and the patient recovered after intravenous injection of fibrinogen. Mathey and his associates (9) described five patients who died of hemorrhage during pulmonary lobectomy or pneumonectomy. In each case, hemorrhage seemed to be due to afibrinogenemia caused by abnormal plasma proteolytic activity.

In the patient described in the present report, the concentration of fibrinogen was sufficiently high to permit clotting to take place. None the less, the patient's whole blood did not clot. The fibrinogen in the patient's plasma appeared to have been digested by the proteolytic enzyme of plasma before clotting occurred. The evidence that fibrinogenolysis actually took place *in vivo* is necessarily indirect. The sample of blood studied was obtained after the patient had been transfused with 3,000 ml. of blood, or almost half the normal circulating blood volume. If the average amount of fibrinogen in this transfused blood was 200 mg. per 100 ml. of plasma, an amount considerably less than the average of normal blood, then the specimen of blood which was studied should have contained almost 100 mg. of fibrinogen per 100 ml. of plasma from the transfused blood alone. Actually, the concentration in this specimen was about 105 mg. per 100 ml. Therefore, it is likely that the fibrinogen in the patient's blood was being destroyed or removed during life. A specimen of blood drawn agonally contained only 33 mg. of fibrinogen per 100 ml. of plasma, further evidence that the fibrinogen which the patient received from the transfused blood was being destroyed.

The mechanism which resulted in the activation of the plasma proteolytic enzyme in this patient remains unexplained. It has been suggested that the cells of various tissues can activate the precursor of the plasma proteolytic enzyme (15–17). As Macfarlane and Biggs (18) have pointed out, however, the mechanism of activation of this enzyme is most complex. Many diverse substances influence the clot lysis time in the test tube, but the relationship between observations concerning fibrinolysis *in vitro* and *in vivo* is not clear.

The patient also had slight hypoprothrombinemia and thrombocytopenia. Neither defect was of sufficient magnitude to produce her profound hemorrhagic state, but it is possible that they may have played a contributory rôle.

The patient who has been described had pancreatic carcinoma. The question arises whether the phenomena observed were not due to the action of trypsin which entered the circulation. Pancreatic trypsin will digest both fibrinogen and fibrin. However, trypsin promotes the clotting of oxalated plasma. In none of the experiments performed *in vitro* was there evidence that the patient's oxalated plasma would clot bovine fibrinogen unless thrombin was added. Moreover, human plasma in high dilution inhibits the lytic action of trypsin. Consequently, it seems unlikely that the hypofibrinogenemia observed in this patient was the result of digestion by pancreatic trypsin.

Treatment in the patient under study was ineffective, but the pathogenesis of the hemorrhagic state was not recognized until she had expired. The transfusion of blood provides large amounts of the protease inhibitors of plasma, but there was no evidence that the patient's blood was deficient in these substances. Perhaps the intravenous injection of large amounts of soy bean inhibitor (19) might have arrested the process, but this substance not only inhibits the proteolytic enzyme of plasma (20), but is anticoagulant as well (21), and its use may have substituted one cause of hemorrhage for another.

SUMMARY

A fatal hemorrhagic state was observed in a patient undergoing surgery for carcinoma of the head of the pancreas. The patient's blood was incoagulable despite the presence of appreciable amounts of fibrinogen. Study of the patient's blood indicated that its failure to clot was associated with excessive plasma proteolytic activity.

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THE HEPATIC BLOOD FLOW IN RESTING HYPERTENSIVE PATIENTS BEFORE AND AFTER SPLANCHNICECTOMY [1]

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INTRODUCTION

Studies of blood flow and calculations of peripheral resistance in various vascular regions of hypertensive patients have generally shown the first to be normal and the second to be increased. With the introduction of the bromsulfalein (BSP) extraction method (*2*), it became possible to measure the circulation in the last important unexplored region, namely, the hepatic-portal, which represents the splanchnic system exclusive of the renal and adrenal circuits. The purpose of this paper is to report the estimated hepatic blood flow (EHBF) and the calculated hepatic-portal resistance (HPR) in a group of hypertensive as compared with a group of normotensive persons, and in some of the same hypertensive patients at various intervals after, as compared with before, surgical (usually lumbodorsal) splanchnicectomy.

METHODS

The methods were identical with those already reported in studies on the effects of the upright posture (*3, 4*). EHBF was measured only after all apparatus had been smoothly operating and the subject had been quietly resting (recumbent) for at least 20 minutes. Hepatic-portal resistance (HPR) was calculated simply by dividing the "mean" (one-half systolic plus diastolic) arterial pressure by the EHBF per second.

RESULTS

Table I shows a statistical analysis of EHBF and HPR in 41 hypertensive patients as compared with 21 normotensive persons concurrently studied by identical methods in this laboratory. It is evident that EHBF was not significantly different, whereas arterial pressure and hence HPR were significantly higher in the hypertensive as compared with the normotensive group.

Table II shows that on statistical analysis EHBF in 13 hypertensive patients two weeks after splanchnicectomy was definitely higher, and HPR significantly lower than before operation. However, as Table III demonstrates, in a group of six hypertensive patients the increased EHBF and HPR returned within four to ten months after operation to essentially preoperative levels. Figure 1 is a chart of the results in one hypertensive patient studied before, and two weeks, ten months, and two years after splanchnicectomy. It illustrates the same trends as shown in Tables II and III.

SUMMARY AND CONCLUSIONS

1. Estimated hepatic blood flow (EHBF) is not significantly different and hepatic-portal resistance (HPR) is significantly higher in hypertensive than in normotensive persons.

2. Shortly after splanchnicectomy EHBF is increased and HPR is decreased as compared with

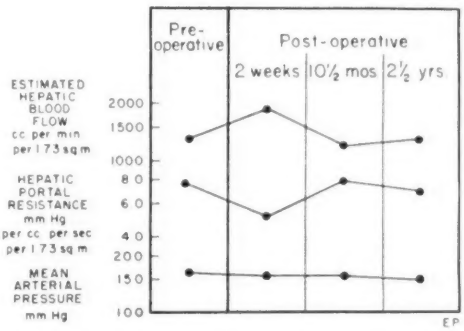

FIG. 1. CHART OF THE EFFECTS OF LUMBODORSAL SPLANCHNICECTOMY ON THE HEPATIC-PORTAL CIRCULATION IN A HYPERTENSIVE PATIENT

[1] Presented in part May 5, 1947 at the Thirty-Ninth Annual Meeting of the American Society for Clinical Investigation, Atlantic City, New Jersey (*1*).

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TABLE I

A comparison between the estimated hepatic blood flow (EHBF), arterial pressure, and hepatic-portal resistance (HPR) of 21 normotensive and 41 hypertensive patients

	EHBF (cc./min./1.73 sq. m.)		Mean arterial pressure (mm. Hg)		HPR (mm. Hg/cc./sec./1.73 sq. m.)	
	Mean	Standard error	Mean	Standard error	Mean	Standard error
Normotensives	1,381	78	100	2	4.7	0.3
Unoperated hypertensives	1,303	50	162	4	8.0	0.4
Difference	−78	85	+62	4	+3.3	0.5
Significance of difference (P*)	0.37		<0.01		<0.01	

* P values of 0.05 or less indicate statistically "significant" differences; values of 0.01 or less are "highly significant."

TABLE II

The early effects of splanchnicectomy upon estimated hepatic blood flow (EHBF), arterial pressure, and hepatic-portal resistance (HPR) in a group of 13 hypertensive patients

	EHBF (cc./min./1.73 sq. m.)		Mean arterial pressure (mm. Hg)		HPR (mm. Hg/cc./sec./1.73 sq. m.)	
	Mean	Standard error	Mean	Standard error	Mean	Standard error
Before operation	1,343	66	154	7	7.1	0.5
Two weeks after operation	1,729	147	144	6	4.7	0.5
Difference	+386	134	−10	6	−2.4	0.6
Significance of difference (P)	0.01		0.13		<0.01	

TABLE III

The late contrasted with the early effects of splanchnicectomy on estimated hepatic blood flow (EHBF), arterial pressure, and hepatic-portal resistance (HPR) in a group of six hypertensive patients

	EHBF (cc./min./1.73 sq. m.)		Mean arterial pressure (mm. Hg)		HPR (mm. Hg/cc./sec./1.73 sq. m.)	
	Mean	Standard error	Mean	Standard error	Mean	Standard error
Before operation	1,288	76	168	7	8.0	0.5
Two weeks* after operation	1,954	227	158	7	5.3	0.8
Four-ten months after operation	1,307	126	157	9	7.4	0.6
Difference between preoperative and early post-operative	+666	158	−11	11	−2.7	0.4
Significance of difference (P)	<0.01		0.36		<0.01	
Difference between preoperative and late post-operative	+19	130	−12	8	−0.5	0.6
Significance of difference (P)	0.89		0.23		0.46	

* This includes one patient studied 9½ weeks after operation.

preoperative measurements in the same hypertensive patients.

3. Within four to ten months after splanchnicectomy EHBF and HPR in hypertensive patients return to their preoperative levels.

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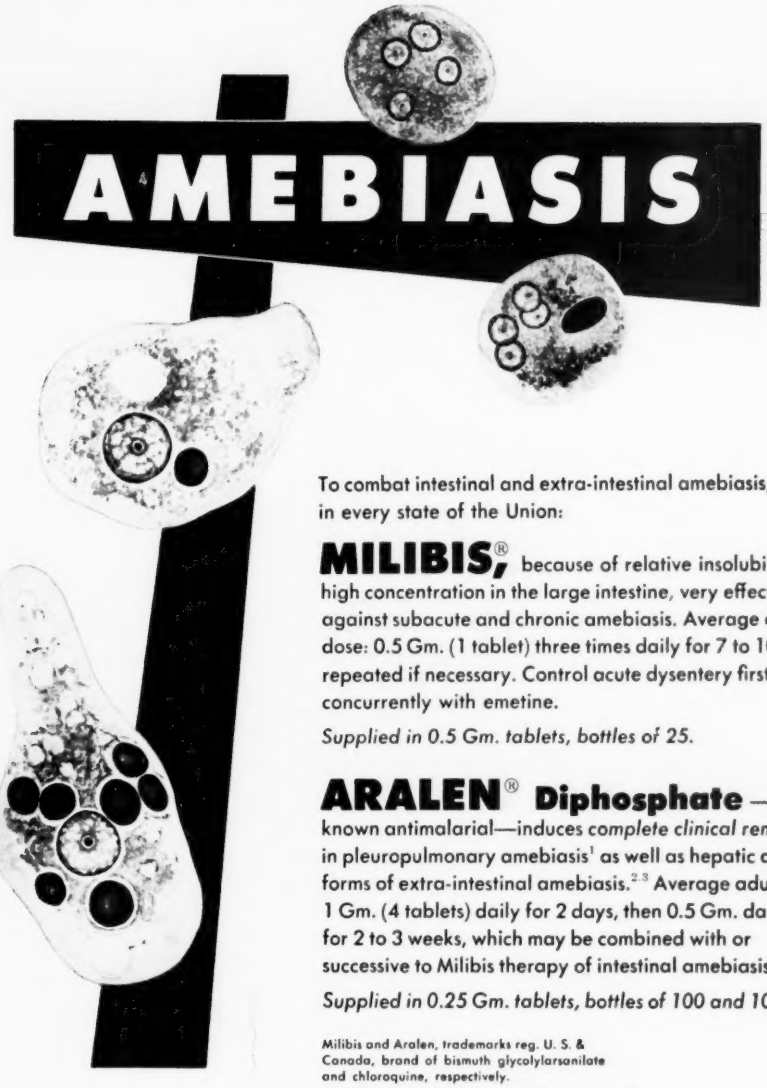

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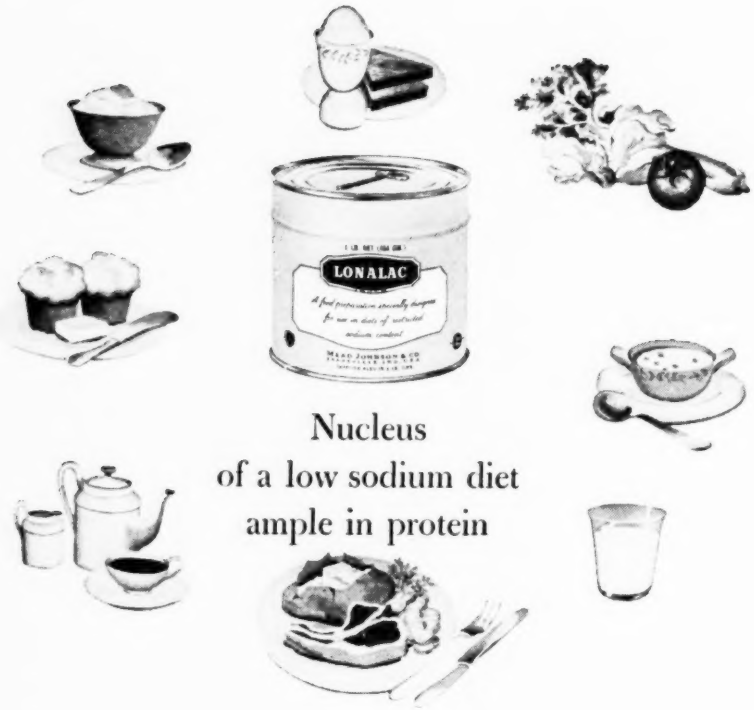

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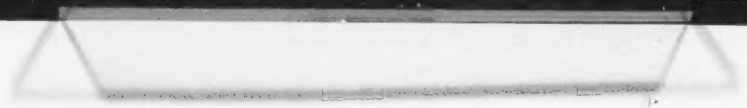

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